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The Short-Term Preservation of Natural Latex

A. S. COOK *

A report is made on the value of various methods of preserving latex for short periods preceding the production of dry rubber by acid coagulation. In particular, studies were made of preservation up to 6 p.m. on the day of tapping and for a period of 24 hours after the addition of preservatives.

There are two normal types of latex preservation: long-term, permitting the shipment of liquid latex which is subsequently processed in the consuming countries, and short-term, to maintain the field latex in a liquid condition for a few hours or days before it is processed into various forms of dry rubber. The usual long-term preservative is ammonia (BISHOP and FULLERTON 1932) but other substances have been used, such as potash (BISHOP and FULLERTON 1932), formalin (ULTEE 1917) and sodium pentachlorphenate (RHODES 1938). Short-term preservatives are commonly known as anticoagulants, and the use of sodium sulphite, ammonia, formalin, sodium carbonate and borax are mentioned in most textbooks, especially by DE VRIES (1920).

The available information on most anticoagulants is not very detailed. The quantities usually given are those which should prevent the precoagulation of latex in the field prior to acid coagulation of the latex at the factory. Anti-coagulants are normally added by the tapper as a dilute aqueous solution (RUBBER RESEARCH INSTITUTE OF MALAYA 1953) before tapping and during collection between 6 a.m. and 11 a.m. The latex is then brought to the factory and coagulated between 11 a.m. and 2 p.m.

The development of centrally placed factories has led to long-distance transport of latex entailing preservation for many hours or even a few days. Attention has thus been drawn to the lack of data on the behaviour of the latex and on the amounts of anticoagulants required. This subject was discussed by the writer at the Rubber Research Institute Latex Symposium 1951 (COOK 1951) and since then by RESING and DE HAAN-HOMANS (1953).

The present paper is divided into three sections. 1. Comparative Tests of Preservatives. 2. Preparation of Smoked Sheet from Latex Preserved until 6 p.m. on the Day of Tapping. 3. Preparation of Smoked Sheet from Latex Preserved for Twenty-Four Hours.

1. COMPARATIVE TESTS OF LATEX PRESERVATIVES

Latex is a complex biological colloid and may undergo spontaneous coagulation by a number of mechanisms (STAMBERGER 1940, PATON 1939, MCMULLEN 1951, ALTMAN 1947, and VAN GILS 1941), the most important of which are probably protein degradation by bacterial and enzymatic attack, acid formation caused by bacteria and the effects of divalent ions. Latex preservatives should therefore have one or more of the following characteristics:

a. bactericidal properties
b. capacity to act as an enzyme inhibitor
c. alkalinity
d. capacity to act as a pH buffer
e. ,, ,, ,, ,, a sequestering agent
f. ,, ,, ,, ,, a colloid stabiliser

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A number of substances or combinations of substances having one or more of the above properties were examined for their effects on various types of latex under different ecological conditions.

Experimental Methods

Latex from various sources, mainly from the Experiment Station of the Rubber Research Institute of Malaya, and from different clones, was tapped and collected as early as possible on dry and wet days, and 10% solutions of the chemicals added at about 10 a.m. to give a range of concentrations of preservative in the latex: samples (2 or more replicates) of latex from each clone were examined on two occasions. Observations were made on different amounts of latex; 20 ml, 200 ml, 1 gallon and 10 gallons. The samples were examined for fluidity by means of a glass rod, for percentage coagulation by volume and for putrefactive odour. Smoked sheets were prepared from some latices. The results given and discussed below are representative of the many observations made.

Results

Details of observations on the effects of single substances are given in the text below; those for sodium metabisulphate and formaldehyde are also set out in Tables 1, 2 and 3. Combinations of chemicals which gave additive preserving effects are also discussed and the results obtained with them are shown in Tables 4, 5 and 6.

Ammonia is a bactericide and an alkali. It also acts in latex as an ion stabiliser and helps to deposit the destabilising magnesium. The unique position it holds as a latex preservative is indicated by the fact that it has been employed in commercial production for over 100 years (NOBLE 1953). For short-term preservation it is normally added, at a level of about 0.3%, before centrifuging. This latex putrefies after a few days; if only 0.2% is added, putrefaction may develop earlier and the latex may coagulate in two or three days. For sheet production, 0.01 to 0.03% is recommended (RUBBER RESEARCH INSTITUTE OF MALAYA 1953) and latex so treated remains liquid for 3 to 10 hours longer than untreated latex.

In our experiments considerable variability was found, depending on the type of latex and on whether the trees were wet or dry. Under most conditions 0.05% ammonia deferred coagulation until 6 p.m. but for less than 24 hours. Latex containing 0.1% also coagulated within 24 hours, while samples containing 0.15% usually putrefied after about 24 hours and coagulated within 2 days. A concentration of 0.1% ammonia raised the pH to over 9 and extra acid was required for coagulation.

Sodium carbonate, Na_2CO_3, is a mild alkali and a concentration of 0.1% raised the pH of the latex to about 7. Its main effect may be its action as a pH buffer against the development of acids by bacterial attack. It was found that 0.1% had no discernable preserving effect. Latex was preserved for an hour or two by 0.2%, until after 6 p.m. by 0.5% and for about 24 hours by 1%.

In combination with small amounts of ammonia, zinc sulphate, sodium benzoate and trisodium phosphate, sodium carbonate gave preservation for about 2 days.

Borax, $Na_2B_4O_7.10H_2O$, is a mild alkali similar to sodium carbonate in degree of alkalinity. It has some bactericidal action and is used as a food preservative; latex was preserved for an hour or two with 0.1%, until about 4 p.m. with 0.2% and until after 6 p.m. with 0.5%. Latex containing 1.0% was liquid after 24 hours but putrefaction had developed.

Ammonium and sodium phosphates are mild alkalis and water softeners. They can associate with proteins (ALEXANDER 1950) and are said to sequester the divalent magnesium ions in latex (PATON 1947). The following phosphates gave no effective preservation up to the 0.5% level: monammonium phosphate, $NH_4(H_2)PO_4$; diammonium phosphate, $(NH_4)_2HPO_4$; sodium pyrophosphate, $Na_4P_2O_7$; disodium phosphate, Na_2HPO_4; and various sodium metaphosphates, $(NaPO_3)n$. Trisodium phosphate, Na_3PO_4,

had some preserving effect; 0.5% delayed coagulation until after 6 p.m. and 1% kept the latex in a liquid condition for more than 24 hours, after which the latex slowly putrefied and coagulated in 2 or 3 days. With the addition of 0.1% zinc sulphate putrefaction was prevented, and with 0.1% ammonia the latex was preserved for 5 or 6 days.

Sodium silicates are mild alkalis, bactericides and pH buffers; they react with proteins (Walker 1953) and have been used as emulsifying and deflocculating agents. Results were especially erratic but with water glass, $Na_2Si_4O_9$, and sodium metasilicate, $Na_2SiO_3.5H_2O$, latex containing 0.5% was preserved for 2 or 3 days.

The preserving effect of water glass was slightly enhanced by 0.1% zinc sulphate and 0.1% sodium benzoate.

Sodium sulphite, Na_2SO_3, has been widely used for many years as an anticoagulant for latex; it is a mild alkali. A concentration of 0.05% is recommended for sheet production (Rubber Research Institute of Malaya 1953) and this amount preserved latex until about 4 p.m. Latex containing 0.1% remained liquid until after 6 p.m. and between 0.5% and 1% was required to keep latex for 24 hours, after which putrefaction developed. Putrefaction was prevented by sodium benzoate and preservation was enhanced by the addition of 0.1% zinc sulphate.

Sodium metabisulphite, $Na_2S_2O_5$, is a mild bactericide, a reducing agent which prevents darkening and is slightly acidic. Adding 1.0% to latex reduced the pH from 6.6 to 5.9. It is used (0.15–0.2%) as an antioxidant during crepe production and although it is not normally suggested as an anticoagulant, preservation has sometimes been observed during crepe production. Observations given in Table 1 show that many clonal latices were preserved with about 0.2% and that yellow fractions separated out leaving a more stable white fraction. The results were erratic and, in many larger scale trials with mixed latex, samples containing 0.1 to 0.5% coagulated within 24 hours. The preserving effect was decreased in wet weather.

It is suggested that in certain instances, sodium metabisulphate might be useful as a short-term preservative during crepe production, in cases when other anticoagulants cannot be employed and when initial coagulation of a yellow fraction is an advantage rather than a hindrance.

Sodium benzoate, C_6H_5COONa, is a bactericide, and has been used in Indonesia to preserve coagulum during transport (Schoon and Zeehuisen 1953). Latex containing 0.2% remained liquid for 1 or 2 hours longer than the untreated control and the development of putrefaction was prevented for more than 24 hours in the coagulum. With 0.5–1.0%, a yellow flocculate was precipitated almost immediately and the remaining white fraction was kept liquid until after 6 p.m. but for less than 24 hours. Preservation for more than 24 hours was obtained by the addition of sodium carbonate, trisodium phosphate, sodium silicate, sodium sulphite and zinc sulphate.

Sodium citrate, $C_6H_5O_7Na_3.2H_2O$, is slightly alkaline in solution and is a pH buffer.

With 0.5% there was little effect on latex, which kept liquid for 2 or 3 hours longer than control.

During an investigation on the lutoids in latex it was observed that when latex was tapped into a sodium citrate solution to give 0.5% in the latex, no large agglomerates were found under the microscope, as described by Homans and van Gils (1948), but there were large numbers of spherical particles about two or three times the size of the average visible rubber particles.

Zinc sulphate, $ZnSO_4.7H_2O$, is a disinfectant and preserving agent. It is stated to be toxic to latex bacteria (Corbet 1929) and is acidic in solution. Latex treated with zinc sulphate immediately thickened and gradually formed a paste but putrefaction was prevented for at least 24 hours. This thickening was prevented by the presence of sodium carbonate, sodium phosphate, sodium silicate, sodium sulphite and sodium benzoate and ammonia, i.e. by alkalis, and preservation for more than 24 hours was obtained in this way.

Formaldehyde, HCHO, has a high chemical reactivity and is used in many industries as a bactericide, preservative, disinfectant and tanning agent (WALKER 1953). In latex it probably acts as a bactericide and perhaps as an enzyme inhibitor; it may also react with latex proteins including those associated with the rubber particles, increasing their resistance to chemical reagents and enzymes. Latex preserved with 2 or 3% formaldehyde has been shipped but the latex thickened and flocculated after some months (WALKER 1953). About 0.01 or 0.02% is recommended as an anticoagulant during latex collection. (RUBBER RESEARCH INSTITUTE OF MALAYA 1953)

Formaldehyde is available commercially as a 38% solution and slowly develops formic acid on storage. In these experiments the solution was neutralised with sodium hydroxide before use.

In the mixed clonal and seedling latex from dry trees, 0.05% preserved the latex for 2 to 4 days and 0.1% for about 10 days. Latex from wet trees was not so readily preserved and one 10-gallon sample containing 0.1% partially coagulated within 24 hours. Good quality smoked sheets were usually prepared from latex containing 0.1% but only occasionally from latex containing 0.05%. No extra acid appeared to be required for coagulation.

Clonal latices showed considerable variation in preservation and Table 2 gives observations on 200 ml samples of clonal latex containing 0.05%, collected from various fields at the R.R.I. Experiment Station on dry and wet days. In dry weather most latices kept for 3 days but in wet weather the majority flocculated or coagulated after 1 day. It was also found that clonal latex from different areas behaved differently; observations on latex of clone Pil D65 from dry trees in two fields are given in Table 3. This latex was stored in corked test tubes and it was usual to find that latex thus kept was preserved for longer periods than larger samples with access to the atmosphere, presumably owing to a lesser degree of bacterial contamination.

Latex preserved with formaldehyde appeared to deteriorate slowly and continuously. Microscopic flocculation usually occurred on addition of the preservative, followed by slow thickening (and the formation of a surface skin) up to the time of complete coagulation, which took place rapidly. In some cases, an initial coagulum of about 25% was formed, the remaining latex being preserved for some days. This behaviour is similar to that of the yellow and white fractions described by HOMANS and VAN GILS (1948). Experiments with fractions separated by centrifuging latex containing 0.1% formaldehyde showed the yellow fraction to be more susceptible than the white fraction to natural flocculation and coagulation. Mild alkalis, such as sodium carbonate, delayed the natural flocculation of the yellow fraction.

To summarise: 0.05–0.2% formaldehyde kept latex in a liquid condition for a few days. The amount required depended on the clonal type of latex, weather conditions and other ecological variants.

Formaldehyde with other chemicals. As mentioned previously, small amounts of formaldehyde are probably rapidly absorbed by the latex proteins and it seemed likely that the presence of alkalis, sequestering agents and possibly bactericides would improve the preservation. Data presented in Table 5 indicate that sodium sulphite, sodium metabisulphite and zinc sulphate destabilised latex containing formaldehyde. Increased preservation was obtained with the mild bactericides sodium benzoate and sodium citrate and with the alkali salts sodium hydroxide and sodium carbonate.

Varied and erratic results were obtained with phosphates (Table 5). Sodium pyrophosphate destabilised the latex at 0.2% and stabilised at 0.5%, whereas disodium and diammonium phosphates increased the preservation at 0.2% and destabilised the latex at 0.5%. Results with trisodium phosphate were erratic but enhanced preservation was obtained in many instances. It seems likely that there is an optimum at different levels for different phosphates. An optimum phosphate content

for sequestration of the magnesium in latex has been discussed by Madge, Collier and Peel (1950). Phosphates appeared to act by preventing the gradual thickening before complete coagulation.

Silicates, 0.1 to 0.5%, in many instances gave large increases in preservation time (Table 5), often for more than a month. The bulk of the latex remained fluid but a skin formed on the surface after a few days. Liquid yellow fractions almost invariably settled out, and they sometimes coagulated within a few days.

Although ammonia and formaldehyde react in aqueous solution to give hexamethylene tetramine, which does not preserve latex, separate addition gave enhanced preservation. Some typical results are shown in Table 6. The longest preservation was obtained when small amounts of ammonia were added a short time after the formaldehyde. It appears that the formaldehyde reacted rapidly and the ammonia acted in a separate and additive preserving capacity.

Summary and Discussion

The most effective single chemical examined was formaldehyde which, at a level of 0.05%, maintained many latices in a liquid condition for 2 or 3 days. The pH was not affected and no extra acid was required for coagulation. Although 0.05% ammonia preserved latex till after 6 p.m. on the day of tapping, about 0.2% was required to preserve for 24 hours, when the pH was increased and extra acid was necessary for coagulation.

Sodium sulphite at the 0.1% level preserved latex till after 6 p.m. but 1.0% was required to keep it liquid overnight and even then putrefaction developed after 24 hours. In certain latices, 0.2% sodium metabisulphite preserved for 24 hours but a yellow fraction was often coagulated.

Mild alkali salts such as sodium carbonate, borax and phosphates preserved latex until 6 p.m. at a concentration of 0.5% and for 24 hours at 1%, but silicates, also mild alkali salts, kept the latex liquid for 2 or 3 days when used at the 0.5% level.

Other bactericides had only slight effects. Sodium benzoate and zinc sulphate delayed the development of putrefaction in the coagulum for more than 24 hours and sodium citrate had a buffering effect for a few hours.

Combinations of these substances with one another often enhanced the preservation, for example: two bactericides; a bactericide with an alkali; a bactericide or an alkali with a sequestering agent; two alkalis. Apart from formaldehyde, only a few combinations of the others preserved latex for much more than 24 hours, at the same time keeping the latex neutral or slightly alkaline:

0.1% zinc sulphate with 0.5% sodium sulphite

0.1% zinc sulphate with 0.5% trisodium phosphate

0.1% sodium benzoate with 0.5% sodium silicate

0.1% ammonia with 0.5% trisodium phosphate

0.1% ammonia with 0.5% sodium silicate

For longer preservation, 0.05% formaldehyde was required with small quantities of alkalis and sequestering agents, such as ammonia, sodium hydroxide, sodium carbonate, sodium phosphates and sodium silicates.

From both the economic and technical viewpoints, quantities of chemicals above 0.5% in the latex are not suitable as short term preservatives for dry rubber production. Alkaline latex requires extra acid for coagulation and costs are thus increased. These factors limit the choice, among the chemicals examined, to sodium sulphite for its cheapness and to small quantities of ammonia and formaldehyde (with or without the addition of cheap alkalis) for their efficacy. Sodium metabisulphite may be useful in crepe production. If latex is to be kept for 24 hours or more before coagulation, the only suitable preservative would appear to be formaldehyde, possibly with the addition of alkalis.

2. THE PREPARATION OF SMOKED SHEET FROM LATEX PRESERVED UNTIL 6 P.M. ON THE DAY OF TAPPING

From Section 1 it will be seen that sodium sulphite, small amounts of ammonia, formal-

dehyde with or without alkalis and possibly sodium metabisulphite, appear to be suitable substances for short-term preservation till the evening of the day of tapping. An investigation was made to determine the minimum amounts of these substances to keep latex until 6 p.m. and to give good quality sheet. The hour chosen is 4–6 hours after the normal time of spontaneous coagulation; it permits observations and coagulation to be effected during the last hour of daylight and it would enable transportation over about 100 miles. The investigation was continued for 15 months, from February 1953 to May 1954, and latex was collected every day where practicable except on Sundays, on other holidays, and when the trees were too wet to be tapped.

Experimental Methods

Samples of latex were taken at the R.R.I. Experiment Station on alternate days. Two types of latex were tapped at about 6.30 a.m. and collected at 10.45 a.m., as is usual in estate practice. These were mixed clonal latex from Field 15 and latex from clone BD 5 in Fields 19 and 24. The trees in Field 15 were twenty-three years old and those in Fields 19 and 24 were twenty-four years old. Both areas were being tapped on the S/2.d/2.100% system. Latex was received at the factory at about noon and sieved through a perforated 8-mesh steel plate to remove lumps, pieces of bark, leaves, etc. The total latex from each area was bulked in a tank, stirred, and a small sample taken for d.r.c. determination. Five-gallon samples were run into small aluminium coagulating tanks ($15\frac{3}{4}$ in. × 18 in. × 16 in. to give ten standard half sheets) and the anti-coagulants added as 10% solutions. A control sample containing no added chemicals was coagulated immediately. The tanks were covered and the condition of the latices examined at 4 p.m. and 6 p.m. After 6 p.m. these were sieved through a 60-mesh wire net, diluted to 15% d.r.c. and coagulated with varying amounts of formic acid. The coagula were flooded with water at 9 p.m. and sheeted next morning at 7.30 a.m. The sheets were allowed to drip for a few hours, and were then dried and smoked for 5 days between 120°F and 140°F in an R.R.I. tunnel-type smoke house. The smoked sheets were examined visually and in some cases by technical testing. Grading was attempted by the standards issued by the Rubber Manufacturers' Association of America.

The aim was to examine each concentration of anticoagulant in each latex, on dry and wet days, and to repeat at least once; but this was not possible during wintering because of the shorter period involved. Trees were regarded as 'wet' if more than 0.2 inches of rain had fallen during the 24 hours prior to tapping.

Results

The two types of latex were chosen mainly for reasons of practical convenience and because it was thought that the BD 5 latex would have a much greater tendency to pre-coagulation. However, it was found that the mixed clonal latex from Field 15 coagulated spontaneously between 1 p.m. and 4 p.m. while the BD 5 latex usually remained liquid until 4 p.m., except during wintering. Good quality smoked sheets were prepared from BD 5 latex on coagulation at 4 p.m. and one sample of untreated latex remained liquid till 6 p.m., but this was exceptional.

The d.r.c. of both types of latex was higher than normal for field latex. No definite effects of wet weather could be established except when rain diluted the latex directly. There was an increase of d.r.c. during wintering. Under normal conditions BD 5 latex had a mean d.r.c. of 42.15% (standard deviation 0.66) and during wintering a mean of 43.19% (standard deviation 0.65). The d.r.c. of the mixed clonal latex slowly increased during the period from about 40% to about 43% with a mean value of 41.50%; during wintering the mean was 43.33% (standard deviation 0.81).

Summaries of the results obtained with sodium sulphite, sodium metabisulphite, ammonia and formaldehyde are given in Tables 7 and 8, and are discussed below. In general, wet weather and wintering caused changes in the latex requiring larger quantities of chemicals for preservation.

Sodium sulphite. About 0.08% was required to preserve the latices from wet and dry trees till 6 p.m. and it was possible to use less on BD 5 latex in dry weather; but during wintering more was required, between 0.1 and 0.15%. Although the latex was maintained in a liquid condition, even latex containing 0.15% gave many Grade 2 sheets owing to the presence of small pin-head blemishes. Varying the amount of acid for coagulation did not prevent this defect. The sheets were light in colour.

When rubber sheet containing these pin-head blemishes was dissolved in benzene, the blemishes showed up as small gas bubbles. By testing with lead acetate paper it was shown that hydrogen sulphide was evolved from latex about 5 hours after tapping, and the presence of 0.1% sodium sulphite lessened but did not prevent gas formation. This was arrested however, by 0.1% formaldehyde. Only a little hydrogen sulphide was evolved when the latex was kept in a closed container into which the contaminating atmosphere had no access. It seems likely that the pin-head bubbles in the sheet were caused by acid coagulation of the latex entrapping gas bubbles produced by fermentation due to contaminating bacteria. Some confirmation of this was obtained by making thin sheet from heat-sterilised latex inoculated with an H_2S producing bacterium, and acid-coagulating after 5 hours. Many more bubbles were found in the dried sheet than in sheet from uninoculated latex.

Other anticoagulant and bactericidal substances were added with the sodium sulphite and to the sulphite latex at 6 p.m. in an attempt to prevent bubble formation: 0.05% sodium carbonate was effective under normal conditions but was ineffective during wintering. Small amounts of formaldehyde (0.03%) prevented bubbles under normal conditions, when added at the same time as the sulphite or at 6 p.m. but bactericides such as sodium pentachlorphenate and zinc sulphate in small amounts did not. During wintering latex containing as much as 0.15% sulphite with 0.5% formaldehyde coagulated in some instances before 6 p.m. There is some evidence to suggest that bubble formation was prevented by adding the second anticoagulant at 6 p.m., thereby counteracting incipient fermentation.

Sodium metabisulphite. Smoked sheet could be produced from latex preserved till 6 p.m. with about 0.15% metabisulphite. The sheets were similar to those from sulphite latex in that many contained fermentation bubbles. There was the additional complication that in some cases rough and patchy sheets were obtained, possibly due to flocculation of the yellow fraction in the latex before acid coagulation.

Ammonia. About 0.06% was required to give good quality sheet in dry and wet weather and this concentration may also be sufficient during wintering. Increased acid was necessary for coagulation, and with 0.06% ammonia about twice the normal quantity was used. The sheets obtained were darker than usual.

Formaldehyde. A concentration of 0.06–0.09% was necessary, depending on the type of latex and the weather. Similar quantities were sufficient during wintering. Extra acid was not required for coagulation. The sheets produced were dark in appearance.

Less formaldehyde could be employed if alkalis were also added to the latex. For example, 0.03% formaldehyde with about the same amount of ammonia gave preservation, but extra acid had to be used for coagulation. Formaldehyde at a level of 0.03–0.05%, combined with about 0.05% sodium carbonate gave adequate preservation and no extra acid was needed.

Effect of Transportation. Five-gallon samples of latex, collected from dry trees during August in the non-wintering period, were treated with 0.07% sodium sulphite, 0.05% ammonia, 0.06% formaldehyde, 0.02% formaldehyde with 0.03% sodium carbonate, placed in closed containers and driven on a lorry for about 50 miles over main roads before being coagulated at 6 p.m. The shaking treatment did not affect the quality of the smoked sheet.

Technical properties of sheet. In a number of trials, 4 sheets from each treatment were tested for Mooney viscosity in the raw state and, after curing in the A.C.S. 1 mix for 40 minutes at 141°C, for tension strength and modulus at 660% elongation. Modulus was also determined after bomb ageing in oxygen at 300 lb sq. in. for 5 days at 70°C. The averaged results given in Table 9 are typical of those obtained. Significant differences between two results are 3 units of Mooney viscosity, 4 kg/sq. cm for modulus and 15 kg/sq. cm for tension strength. These results show that sodium sulphite had very little effect, ammonia slightly affected the ageing properties, and formaldehyde slightly reduced the tension strength before ageing and the modulus before and after ageing.

Discussion

The amounts of sodium sulphite, ammonia, formaldehyde and formaldehyde with alkalis necessary to preserve latex till 6 p.m. on the day of tapping for the production of good quality smoked sheet have been established. The use of sodium metabisulphite is suspect because of the possibility of flocculation of the yellow fraction. Sodium sulphite is the cheapest of these anticoagulants, but the formation of fermentation bubbles generally gives a second grade sheet, judged by the usual visual standards. Under non-wintering conditions the defect can usually be prevented by the addition of small amounts of sodium carbonate or formaldehyde to the latex. Ammonia gas, at the time of writing, is twice the price of sodium sulphite, and formaldehyde, calculated as the pure chemical in formalin solution, three times the price. Both ammonia and formaldehyde give top quality sheet, but ammonia has the disadvantage of requiring extra acid for coagulation. Formaldehyde would appear to be the most useful preservative on the grounds of cost and quality of sheet, especially if the amount of formaldehyde is reduced by the addition of sodium carbonate which is one-tenth the price of formaldehyde. The small quantities of these chemicals required had only minor effects on the technical properties of the sheet.

Within the range of quantities examined, the variations in the amounts of preservatives necessary for keeping latex was quite large, depending on the type of latex, the weather and the season of the year. It is possible that some types of latex, produced on different soils and with lower d.r.c., would require only small amounts of preservatives, while others may require the maximum quantities discussed. In practice, it would be advisable to determine, for each source of latex to be separately bulked, the smallest amounts needed for safe handling during wet weather and in the wintering period.

3. THE PREPARATION OF SMOKED SHEET FROM LATEX PRESERVED FOR TWENTY-FOUR HOURS

It was concluded from Section 1 that the most suitable preservative for keeping latex for one day before manufacturing smoked sheet is probably formaldehyde alone or in combination with alkalis. This was further investigated during the period December 1953 to May 1954, which included a wintering season.

The procedures used for preserving the latex and for making the smoked sheet were similar to those described in Section 2, except that the latex was kept for 24 hours after the chemicals were added, and coagulated about noon on the day after tapping. The amount of acid was varied at each concentration of preservative, in order to find the optimum amount to give the best quality of smoked sheet. Two sources of latex at the R.R.I. Experiment Station were used: an area of mixed clonal latex from Field 15 different from that used in Section 2; and an area of mixed clonal and seedling latex from Field 2. The chemicals added were 0.05 – 0.2% formaldehyde alone or in combination with 0.05% and 0.1% sodium carbonate, sodium metasilicate and trisodium phosphate. Drying of the sheeted coagula was continued for more than 5 days if necessary, until most of the sheets were dry.

Results

The d.r.c. of both types of latex were over 42% and increased during the wintering period. The mixed clonal latex from Field 15 had a mean d.r.c. of 42.18% (s.d. 0.06) before wintering and 43.45% (s.d. 0.79) during wintering. The comparable d.r.c. for Field 2 latex were 42.25% (s.d. 0.27) before wintering and 42.96% (s.d. 0.73) during wintering.

In all cases, even with 0.05% formaldehyde, the latices were in liquid condition after 24 hours. Latices containing 0.05 – 0.15% formaldehyde were more viscous than normal but this had no effect on tractability or coagulation after dilution. Addition of the alkalis rendered the latex less viscous than fresh latex. Wet weather and wintering had only slight deleterious effects on the quality of the sheet made from latex containing 0.05% formaldehyde; 0.1% formaldehyde masked any of these effects.

Little difference was found in the behaviour of the two types of latex except that, Field 15 latex generally required more acid for coagulation than did Field 2 latex. The optimum amount of acid was found by examining the coagula formed and the dried sheet. Too little acid gave soft coagula and uneven sheets. Too much acid gave tough coagula and small thick sheets which took longer to dry—except that with 0.2% formaldehyde extra acid gave soft coagula and long thin sheets. The latter phenomenon is probably associated with the effect of formaldehyde on the proteins. A summary of the results obtained is given in Table 10.

Although 0.05% formaldehyde kept the latex in a liquid and tractable condition for 24 hours, the sheet produced was often rough and contained fermentation bubbles; it was nevertheless of normal size and dried in the usual time of 5 days, or slightly longer. The use of 0.1% or more formaldehyde enabled sheet of mainly good quality to be made but this concentration produced a smaller and thicker sheet which took 8 or 9 days to dry. With up to 0.15% formaldehyde only the normal quantity of acid was required for coagulation but with 0.2% about a third more acid was needed.

The use of 0.05% sodium carbonate, trisodium phosphate and sodium metasilicate with 0.05% formaldehyde improved the quality of the sheet but did not completely prevent the production of a proportion of rough sheets with some fermentation bubbles; 0.1% sodium carbonate was not much better but 0.1% trisodium phosphate and sodium metasilicate gave good sheet with 0.05% formaldehyde. With 0.1% formaldehyde the addition of the salts had little beneficial effect other than reducing the viscosity, and 0.1% sodium carbonate gave sticky sheets which took slightly longer to dry. Sodium metasilicate appeared to give the best sheets. Extra acid for coagulation was required when the salts were used; sodium carbonate required the most and sodium metasilicate the least.

The optimum amount of acid (as determined in this experiment) gave coagula which appeared normal or slightly hard and most dried sheets were smaller and thicker than usual and took longer than normal to dry. This seemed to be a direct effect of keeping latex in contact with about 0.1% formaldehyde for the relatively long period of 24 hours. Latex containing 0.05% formaldehyde gave smoked sheets which were darker than normal and had dull surfaces. Higher quantities of formaldehyde gave dark sheets with shiny or glossy surfaces and some sheets containing the higher amounts of salts were sticky.

It may be relevant to note here that in a few experiments with latex kept for up to a week before coagulating, more acid was required to produce a compact coagulum.

Technical Properties of Sheet

Smoked sheets from a number of experiments were examined for technical properties and, as described in Section 2, four sheets from each treatment were tested. Averaged results are given in Table 11. Mooney viscocity of the raw rubber (Mooney Viscosity, VR) was not much affected by 0.05% and

0.1% formaldehyde but tension strength and ageing were slightly reduced; sodium carbonate slightly affected modulus ageing.

Discussion

About 0.1% formaldehyde or 0.05% formaldehyde with 0.1% trisodium phosphate or sodium metasilicate were the approximate minimum quantities found to produce good quality smoked sheet from the two latices examined, when these were kept for 24 hours before coagulating. Using normal methods of preparation, a smaller and thicker sheet was produced which took longer to dry than usual. No attempts were made to overcome this by altering details of preparation, e.g. milling to give thinner coagula. Of the salts, sodium carbonate is the cheapest and trisodium phosphate the most expensive but even the cost of the latter is less than half that of formaldehyde; sodium carbonate did not appear to have so much beneficial effect as trisodium phosphate or sodium metasilicate; and sodium metasilicate gave better sheet, required less acid and gave quicker drying than trisodium phosphate. It has been mentioned previously that a liquid yellow fraction settles out from latex containing formaldehyde and sodium metasilicate but this did not appear to affect the preparation of sheet from latex preserved for one day. It would seem therefore that the cheapest and most effective combination would be formaldehyde with sodium metasilicate.

It should be emphasised that the minimum amounts of chemicals required probably vary with different sources of latex and for practical purposes preliminary investigations should be made to determine these minima.

SUMMARY

A number of chemicals were investigated to determine in detail their effects on natural rubber latex, with a view to ascertaining the most economical method of preserving latex for a short period before the production of dry rubber by acid coagulation.

1. The effects on various types of latex of alkalis, sequestering agents, bactericides and combinations of these are discussed in Section 1. Formaldehyde was found to be the most effective chemical in small concentrations although other substances and combinations kept latex in a liquid condition for periods varying from a few hours to several days. Preservation was influenced by the type of latex, by weather and other ecological conditions, and especially by wintering of the trees.

2. Section 2 gives results of investigations on the usefulness of sodium sulphite, sodium metabisulphite, ammonia and formaldehyde in preserving latex (for the production of smoked sheet) up to 6 p.m. on the day of tapping. Weather conditions and wintering were taken into account. For each substance, the quantity required for effective preservation was determined. Formaldehyde appeared to be the most useful preservative for producing high-grade sheet at low cost.

3. Section 3 deals with the production of smoked sheet from latex preserved for 24 hours with formaldehyde alone, or formaldehyde with sodium carbonate, trisodium phosphate or sodium metasilicate. The cheapest and most effective combination appeared to be formaldehyde with sodium metasilicate.

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TABLE 1. EFFECTS OF SODIUM METABISULPHATE ON LATEX

Clonal type and condition of trees when tapped	Amount of sodium metabisulphate in latex (% wt)	Number of days to coagulation	Observation on coagulation
PB 186, dry trees	0.05	1	Complete
„ „ „	0.10	2	Complete
„ „ „	0.15	1 – 3	A 20% yellow fraction coagulated after one day, white fraction remained liquid for four days.
„ „ „	0.20	3	Complete
„ „ „	0.50	4	Complete
PB 186, wet trees	0.05 – 0.20	1	Complete
„ „ „	0.50	2	Partial
AVROS 49, dry trees	0.17 – 0.20	1 – 2	Yellow fraction
AVROS 50 „	0.17	2	Complete
AVROS 152 „	0.17	2	Complete
BD 5 „	0.17	2	Complete
Gl 1 „	0.17	2	Complete
Pil A44 „	0.17	2	Complete
Pil B84 „	0.17	2	Complete
PB 23 „	0.17 – 0.20	1	Yellow fraction or complete coagulation
PB 25 „	0.17	3	Complete
PB 180 „	0.17 – 0.20	1 – 4	Complete
PB 186 „	0.17 – 0.20	2 – 4	Yellow fraction or complete coagulation
RRIM 501 „	0.17 – 0.20	2 – 3	Yellow fraction or complete coagulation
Tjir 1 „	0.17 – 0.20	1 – 2	Complete
Tjir 16 „	0.17	2	Complete

TABLE 2. OBSERVATIONS ON THE PRESERVATION OF CLONAL LATEX WITH 0.05% FORMALDEHYDE

L = liquid T = thick F = flocculated C = coagulated

Clone	Latex collected from dry trees			Latex collected from wet trees		
	Condition of latex after:			Condition of latex after:		
	1 day	2 days	3 days	1 day	2 days	3 days
AVROS 33	L	L	L	L	L	T
AVROS 49	L	L	L	L	L	T
AVROS 50	L	L	L	L	F	C
AVROS 152	L	L	L	L	L	T
BD 5	T	C	—	C	—	—
BD 10	L	L	L	T	T	F
BR 1	T	T	T	L	L	T
BR 2	L	L	L	L	F	F
Gl 1	T	T	T	L	L	L
KH 127	L	T	C	T	T	C
Lun N	L	L	L	L	F	F
PB 23	L	L	L	L	L	L
PB 25	L	L	C	T	T	F
PB 49	T	T	T	F	F	C
PB 123	T	T	C	L	C	—
PB 186	T	C	—	F	F	C
Pil A44	L	L	L	L	L	L
Pil B16	L	L	L	L	L	T
Pil B84	L	C	—	L	F	F
Pil D65	C	—	—	F	C	—
RRIM 501	L	L	L	L	L	F
RRIM 502	L	L	L	L	F	C
RRIM 509	L	C	—	T	F	F
RRIM 514	L	L	L	T	F	F
Rub 426	L	L	T	L	L	L
Sab 4	L	T	T	L	F	C
Tjir 1	L	L	L	L	L	L
Tjir 16	L	L	T	L	L	L
TK 12	L	L	L	L	L	T

TABLE 3. PRESERVATION BY FORMALDEHYDE OF LATEX FROM CLONE PIL D65

Amount of formaldehyde added to latex (% wt)	Latex from Field 17			Latex from Field 31		
	No. of days to formation of surface skin	No. of days to 25% coagulation	No. of days to 100% coagulation	No. of days to formation of surface skin	No. of days to 25% coagulation	No. of days to 100% coagulation
0.05	< 1	< 1	< 1	2	9	10
0.10	< 1	< 1	> 15	3	15	20
0.20	15	> 15	> 15	7	12	16
0.30	> 15	> 15	> 15	15	20	> 25
0.40	> 15	> 15	> 15	> 25	> 25	> 25
0.50	> 15	> 15	> 15	> 25	> 25	> 25

TABLE 4. THE PRESERVING EFFECTS OF COMBINATIONS OF CHEMICALS OTHER THAN FORMALDEHYDE

Chemicals and amounts in latex (% wt)					No. of days to coagulation	Percentage volume of coagulum
0.05	ammonia	+ 0.1	zinc sulphate		1	20
0.05	„	+ 0.5	„	„	1	100
0.10	„	+ 0.1	„	„	2	10
0.10	„	+ 0.5	„	„	2	100
0.05	„	+ 0.1	trisodium	phosphate	1	100
0.05	„	+ 0.5	„	„	2	20
0.10	„	+ 0.1	„	„	2	100
0.10	„	+ 0.5	„	„	6	100
0.05	„	+ 0.2	sodium	silicate	2	100
0.05	„	+ 0.4	„	„	4	100
0.05	„	+ 0.6	„	„	11	100
0.10	„	+ 0.5	„	„	12	100
0.10	„	+ 0.1	sodium	carbonate	1	100
0.10	„	+ 0.5	„	„	2	100
0.10	sodium carbonate	+ 0.1	trisodium	phosphate	1	100
0.10	„ „	+ 0.5	„	„	1	20
0.50	„ „	+ 0.1	„	„	2	30
0.5	„ „	+ 0.5	„	„	2	100
0.10	sodium benzoate	+ 0.2	sodium	sulphite	1	100
0.10	„ „	+ 0.5	„	„	2	100
0.50	„ „	+ 0.2	„	„	1	100
0.50	„ „	+ 0.5	„	„	2	100
0.10	„ „	+ 0.1	sodium	silicate	1	80
0.10	„ „	+ 0.5	„	„	3	10
0.50	„ „	+ 0.1	„	„	2	100
0.50	„ „	+ 0.5	„	„	3	100
0.30	„ „	+ 0.5	trisodium	phosphate	2	50
0.50	„ „	+ 0.5	„	„	1	20
0.10	„ „	+ 0.1	sodium	carbonate	1	30
0.10	„ „	+ 0.5	„	„	1	5
0.50	„ „	+ 0.1	„	„	1	20
0.50	„ „	+ 0.5	„	„	2	20
0.10	„ „	+ 0.1	zinc	sulphate	2	100
0.3	„ „	+ 0.1	„	„	2	100
0.30	„ „	+ 0.5	„	„	2	100
0.10	zinc sulphate	+ 0.1	sodium	sulphite	2	100
0.10	„ „	+ 0.5	„	„	3	100
0.50	„ „	+ 0.1	„	„	1	60
0.50	„ „	+ 0.5	„	„	2	100
0.10	„ „	+ 0.1	sodium	silicate	2	100
0.10	„ „	+ 0.5	„	„	2	100
0.50	„ „	+ 0.1	„	„	1	50
0.50	„ „	+ 0.5	„	„	2	100
0.10	„ „	+ 0.1	trisodium	phosphate	3	100
0.10	„ „	+ 0.5	„	„	3	100
0.50	„ „	+ 0.1	„	„	1	100
0.50	„ „	+ 0.5	„	„	1	100
0.10	„ „	+ 0.1	sodium	carbonate	1	100
0.10	„ „	+ 0.5	„	„	1	100
0.50	„ „	+ 0.1	„	„	2	100
0.50	„ „	+ 0.5	„	„	1	100

TABLE 5. THE PRESERVING EFFECTS OF FORMALDEHYDE WITH OTHER CHEMICALS

Chemicals and amounts in latex (% wt)	Volume of latex samples	No. of days coagulation	Percentage volume of coagulum	Other observations
0.05 formaldehyde	200 ml	4 – 5	80 – 100	—
0.10 „	„	12	30 – 100	
0.05 „ + 0.10 sodium benzoate	„	7	100	
0.05 „ + 0.10 zinc sulphate	„	4	20	—
0.05 „ + 0.50 „ „	„	1	100	—
0.05 „ + 0.10 sodium sulphite	„	1	50	—
0.05 „ + 0.50 „ „	„	1	50	—
0.05 „ + 0.10 sodium metabisulphite	„	1	25	Yellow fraction
0.05 „ + 0.50 „ „	„	1	25	Yellow fraction
0.05 „ + 0.10 sodium hydroxide	„	10	50	—
0.10 „ + 0.10 „ „	„	19	100	—
0.05 „ + 0.10 sodium carbonate	„	7	40 – 100	—
0.05 „ + 0.25 „ „	„	11	30	—
0.05 „ + 0.10 sodium pyrophosphate	„	3	20	—
0.05 „ + 0.20 „ „	„	2	50	—
0.05 „ + 0.50 „ „	„	10	100	—
0.10 „ + 0.50 „ „	„	17	100	—
0.05 „ + 0.10 disodium phosphate	„	8	100	—
0.05 „ + 0.25 „ „	„	7	30	—
0.05 „ + 0.50 „ „	„	3	50	—
0.05 „ + 0.10 trisodium phosphate	„	9	100	—
0.05 „ + 0.20 „ „	„	5	100	—
0.05 „ + 0.10 sodium silicate	„	12	100	—
0.05 „ + 0.25 „ „	„	12	100	—
0.05 „ + 0.10 sodium metasilicate	„	6	40	—
0.05 „ + 0.50 „ „	„	24	100	—
0.05 „	20 ml	10 – 16	25 – 100	—
0.05 „ + 0.10 sodium citrate	„	12 – 23	5 – 100	—
0.05 „ + 0.10 sodium carbonate	„	16 – 23	25 – 100	—
0.05 „ + 0.05 trisodium phosphate	„	18 – 22	100	—
0.05 „ + 0.10 „ „	„	20 – 25	100	—
0.05 „ + 0.20 „ „	„	19 – 21	100	—
0.05 „ + 0.50 „ „	„	14 – 16	100	—
0.05 „ + 0.10 sodium metasilicate	„	26 – 30	100	Many replicates all liquid yellow fractions

TABLE 6. PRESERVATION OF LATEX WITH FORMALDEHYDE AND AMMONIA

Method of addition of chemicals to latex	Formaldehyde added (% wt)	Ammonia added (% wt)	Number of days to spontaneous coagulation
Aqueous solution mixed in stoichiometric amounts before adding to latex	0.10	0.04	< 1
Addition of single substances in solution	0.05	—	3
	—	0.20	3
Ammonia added shortly after formaldehyde	0.05	0.01	7
	0.05	0.02	7
Formaldehyde added shortly after ammonia	0.05	0.20	3
	0.10	0.20	4
Formaldehyde added 24 hours after ammonia	0.05	0.20	5

TABLE 7. SMOKED SHEET FROM LATEX COLLECTED UNDER NON-WINTERING CONDITIONS AND PRESERVED UNTIL 6 P.M. ON THE DAY OF TAPPING

Chemicals added to latex (% wt)	Grade of smoked sheet produced			
	Mixed clonal latex		BD 5 latex	
	Dry trees	Wet trees	Dry trees	Wet trees
0.05 sodium sulphite	Nil *	—	1 and 2	—
0.06 „ „	2 and nil	—	1 and 2	3
0.07 „ „	2	2	1	2
0.08 „ „	1 and 2	1 and 2	1 and 2	1 and 2
0.09 „ „	1 and 2	1 and 2	Mainly 1	1 and 2
0.10 „ „	1 and 2	1 and 2	Mainly 1	1 and 2
0.12 „ „	1 and 2	1 and 2	—	1 and 2
0.15 „ „	1 and 2	1 and 2	—	1 and 2
0.10 sodium metabisulphite	1 and nil	1, 2 and nil	1, 2 and 3	1 and 2
0.15 „ „	1 and 2	1 and 2	1, 2 and 3	1 and 2
0.02 ammonia	Nil	—	—	—
0.03 „	Mainly 2	—	2	—
0.04 „	Mainly 1	1	1, 2 and nil	Nil
0.05 „	1 and 2	1 and 2	Mainly 1	1, 2 and nil
0.06 „	Mainly 1	Mainly 1	Mainly 1	Mainly 1
0.02 formaldehyde	2 and nil	Nil	—	—
0.03 „	—	—	2 and 3	2
0.04 „	2 and nil	—	1 and 2	2
0.05 „	1 and nil	1 and 2	1 and 2	Mainly 2
0.06 „	1	1 and 2	1 and 2	Mainly 2
0.07 „	1	—	1	1 and 2
0.08 „	—	Mainly 1	—	1
0.09 „	—	1	—	—
0.10 „	1	1	1	1
0.08 sodium sulphite + 0.20 sodium carbonate	1 and 2	—	2	—
0.08 „ „ + 0.05 „ „	1	Mainly 1	1	Mainly 1
0.08 „ „ + 0.02 formaldehyde	1	1 and 2	1	1
0.08 „ „ + 0.03 „	1	1	1	1
0.08 „ „ + 0.01 „ (at 6 p.m.)	Mainly 2	1 and 2	Mainly 1	—
0.08 „ „ + 0.02 „ (at 6 p.m.)	1 and 2	1 and 2	1	1
0.08 „ „ + 0.03 „ (at 6 p.m.)	1	1	1	1
0.08 „ „ + 0.02 sodium pentachlorphenate	Mainly 2	2	1 and 2	2
0.08 „ „ + 0.02 „ „ (at 6 p.m.)	Mainly 2	2	1	1 and 2
0.08 „ „ + 0.02 zinc sulphate	2	—	1 and 2	—
0.08 „ „ + 0.02 „ „ (at 6 p.m.)	1 and 2	—	Mainly 1	—
0.02 formaldehyde + 0.02 ammonia	1, 2 and nil*	2 and nil	2 and nil	—
0.02 „ + 0.03 „	1	1 and nil	1 and 2	1, 2 and nil
0.03 „ + 0.02 „	1 and 2	1 and 2	1, 2 and nil	3
0.03 „ + 0.03 „	1	1 and 2	1 and 2	2
0.03 „ + 0.04 „	—	1	—	1
0.04 „ + 0.02 „	—	1	—	1
0.02 „ + 0.03 sodium carbonate	Mainly 2	2	1 and nil	—
0.02 „ + 0.05 „ „	1 and 2	2	Mainly 1	Mainly 2
0.03 „ + 0.03 „ „	Mainly 1	Mainly 2	1 and 2	2
0.03 „ + 0.05 „ „	1	1 and 2	Mainly 2	2
0.04 „ + 0.03 „ „	1	1	1 and 2	2
0.04 „ + 0.05 „ „	1	1	1 and 2	2
0.05 „ + 0.05 „ „	—	1	Mainly 1	1

* 'nil' indicates that in some or all of the trials latex coagulated spontaneously before 6 p.m. and thus could not be converted into sheet.

TABLE 8. SMOKED SHEET FROM LATEX COLLECTED UNDER WINTERING CONDITIONS AND PRESERVED UNTIL 6 P.M. ON THE DAY OF TAPPING

Chemicals added to latex (% wt)	Grade of smoked sheet produced			
	Mixed clonal latex		BD 5 latex	
	Dry trees	Wet trees	Dry trees	Wet trees
0.05 sodium sulphite	—	—	Nil	Nil
0.06 „ „	—	—	Nil	—
0.07 „ „	—	—	1	—
0.08 „ „	Nil	Nil	1, 2 and nil	2 and nil
0.15 „ „	—	1	—	1 and 2
0.03 ammonia	Nil	—	Nil	Nil
0.04 „	—	—	Nil	—
0.05 „	—	—	1	—
0.06 „	1	—	—	—
0.02 formaldehyde	—	—	Nil	Nil
0.06 „	1	—	—	—
0.07 „	—	1 and nil	1	—
0.08 „	—	1	1	—
0.09 „	—	1	—	—
0.10 „	1	1	1	1
0.08 sodium sulphate + 0.05 sodium carbonate	2	—	1 and 2	1 and 2
0.08 „ „ + 0.02 formaldehyde	Nil	—	1	—
0.10 „ „ + 0.05 „	—	2 and nil	1 and 2	—
0.15 „ „ + 0.05 „	—	2 and nil	1 and 2	—
0.10 „ „ + 0.03 „ (at 6 p.m.)	—	2 and nil	—	1
0.10 „ „ + 0.05 „ (at 6 p.m.)	—	Nil	1 and nil	—
0.15 „ „ + 0.05 „ (at 6 p.m.)	—	Nil	1, 2 and nil	—
0.08 „ „ + 0.03 sodium pentachlorphenate (at 6 p.m.)	Nil	—	1 and 2	—
0.08 „ „ + 0.03 „ (at 6 p.m.)	1, 2 and nil	—	1 and 2	—
0.08 „ „ + 0.03 zinc sulphate	2	—	Mainly 2	—
0.08 „ „ + 0.03 „ (at 6 p.m.)	2	—	Mainly 2	—
0.08 „ „ + 0.05 borax	Nil	—	2	—

TABLE 9. TECHNICAL PROPERTIES OF SMOKED SHEET IN A.C.S. NO. 1 MIX MADE FROM LATEX PRESERVED UNTIL 6 P.M. ON THE DAY OF TAPPING

Chemicals added to latex (% wt)	Mooney viscosity (V_R)	Tension strength (kg/sq. cm)		Modulus at 660% elongation (kg/sq. cm)	
		Before ageing	After ageing	Before ageing	After ageing
Nil (coagulated at noon)	88	157	117	47	86
0.08 sodium sulphite	89	155	116	44	84
0.08 „ „ + 0.03 formaldehyde	87	145	117	44	75
0.08 „ „ + 0.03 sodium pentachlorphenate	87	146	120	40	79
0.08 „ „ + 0.05 sodium carbonate	88	158	136	47	89
0.08 „ „ + 0.05 borax	86	147	101	41	87
0.06 ammonia	88	159	96	45	72
0.06 formaldehyde	87	141	109	42	71
0.10 „	86	137	110	42	65
0.02 „ + 0.03 ammonia	87	158	107	48	79
0.02 „ + 0.05 sodium carbonate	88	151	118	46	86

TABLE 10. SMOKED SHEET FROM LATEX PRESERVED FOR 24 HOURS

Chemicals added to latex (% wt)	Formic acid for coagulation (fl. oz. per 100 gal. at 15% d.r.c.)	Drying time (days)	Appearance of sheet	Grade of sheet
0.05 formaldehyde	8	5 – 8	Fairly normal, some rough and some bubbles	1 and 2
0.10 „	8	8 – 9	Some smaller, a few bubbles	Mainly 1
0.15 „	8	8 – 10	Some smaller, shiny	1
0.20 „	11	8 – 9	Some smaller, shiny	1
0.05 „ + 0.05 sodium carbonate	8	5 – 8	Normal, mainly good, but a few bubbles and some slightly rough	Mainly 1
0.10 „ + 0.05 „ „	9	8 – 9	Some smaller and shiny, a few bubbles and slightly rough	Mainly 1
0.05 „ + 0.10 „ „	10	8 – 9	Some smaller and shiny, some bubbles and slightly rough	1 and 2
0.10 „ + 0.10 „ „	13	8 – 10	Smaller, shiny, sticky, a few bubbles	1 and 2
0.05 „ + 0.05 trisodium phosphate	8 (Field 2)	5 – 8	Normal, a few bubbles and slightly rough	Mainly 1
0.05 „ + 0.05 „ „	9 – 10 (Field 15)	5 – 8	Normal, a few bubbles and some rough	1 and 2
0.10 „ + 0.05 „ „	8 – 9	7 – 9	A few smaller, a few bubbles and slightly rough	Mainly 1
0.05 „ + 0.10 „ „	10 – 11	8 – 9	Some smaller, good	1
0.10 „ + 0.10 „ „	10 – 11	8 – 10	Smaller, shiny, some rough	Mainly 1
0.05 „ + 0.05 sodium metasilicate	8 – 9	5 – 8	Normal, some rough and some bubbles	Mainly 1
0.10 „ + 0.05 „ „	8 – 9	8 – 9	Smaller, shiny, good	1
0.50 „ + 0.10 „ „	8 – 10	5 – 8	Mainly normal, good	1
0.10 „ + 0.10 „ „	8 – 10	8 – 9	Smaller, shiny, good	1

TABLE 11. TECHNICAL PROPERTIES OF SMOKED SHEET IN A.C.S. NO. 1 MIX FROM LATEX PRESERVED FOR 24 HOURS

Chemicals added to latex (% wt)	Mooney viscosity (V_R)	Tension strength (kg/sq. cm)		Modulus at 660% elongation (kg/sq. cm)	
		Before ageing	After ageing	Before ageing	After ageing
Nil (coagulated at noon on day of tapping)	90	167	121	51	90
0.05 formaldehyde	86	151	133	47	76
0.01 „	87	151	110	45	76
0.05 „ + 0.05 sodium carbonate	91	145	128	48	74
0.05 „ + 0.10 „ „	91	153	85	44	72
0.10 „ + 0.05 „ „	89	149	112	40	69
0.10 „ + 0.10 „ „	89	153	84	43	71
0.05 „ + 0.05 trisodium phosphate	90	151	128	44	77
0.05 „ + 0.10 „ „	91	159	116	46	73
0.10 „ + 0.05 „ „	87	154	114	46	76
0.10 „ + 0.10 „ „	88	149	110	45	76
0.05 „ + 0.05 sodium metasilicate	90	150	108	51	77
0.05 „ + 0.10 „ „	89	148	104	49	79
0.10 „ + 0.05 „ „	88	148	93	47	77
0.10 „ + 0.10 „ „	88	151	95	47	79

Vegetation of Rubber Plantations

P. R. WYCHERLEY

The results of surveys of the ground cover vegetation on rubber estates are reviewed. There are indications that prolonged Hevea cultivation with modern upkeep methods has impoverished the flora and led to the predominance in the vegetation of types believed to be less beneficial than the displaced species to the growth of Hevea.*

Early experience of tree crops in tropical plantations showed that serious soil deterioration and erosion could take place if the ground was not allowed to develop a cover of vegetation. This cover is very important both before and after a complete canopy is formed by the crowns of trees. The cover may be beneficial in nutrient cycles at all stages, and it remains essential after the canopy is formed, for the control of erosion on slopes.

It has become evident that the cover of vegetation strongly influences the growth and performance of *Hevea brasiliensis*. The grass *Imperata cylindrica (lalang)* and the fern *Gleichenia linearis (resam)* suppress the growth of *Hevea* so severely that death may result (RUBBER RESEARCH INSTITUTE OF MALAYA 1938). On the other hand, leguminous cover plants afford good protection to the soil, often improving it, without seriously competing with the trees. There are however, many species occurring in the ground flora about whose individual worth little is known.

Until recently there have been relatively few trials in Malaya comparing the effects of pure stands of the various indigenous and introduced species. Further trials of this nature have now been started. In order to discover which species are worth investigating, botanical surveys carried out by the R.R.I.M. have been reviewed and are reported here.

From an analysis of these surveys it is possible to detect certain trends in the development of flora under the influence of rubber cultivation practices and these trends are also discussed in this paper.

In certain experiments, such as that conducted in Field 27 of the R.R.I. Experiment Station (R.R.I. PLANTERS' CONFERENCE 1938; RUBBER RESEARCH INSTITUTE OF MALAYA 1940; RUBBER RESEARCH INSTITUTE OF MALAYA 1935 – 1951), it was found that a mixed association of indigenous species was relatively easy to establish after clearing forest land; moreover, this was maintained at little cost by slashing and persisted as a natural complex both in the open and under shade, although floristic changes took place. Such an association protected the soil and permitted growth of *Hevea* as good as, and sometimes better than that obtained with sown leguminous covers. Associations of the creeping fern *Nephrolepis biserrata*, deep-rooting woody species and soft leaved scandent plants were found most suitable.

Less clearing of forest for new rubber is now being done in Malaya. Some lalang and other degraded lands are being planted, but present-day planting activities are mainly concentrated on replanting. Areas being replanted are often in the oldest parts of estates and are usually isolated from forest; perhaps for this reason the invasion by some species is reduced. In such cases it is customary to establish creeping legumes which, apart from being susceptible to drift from sodium arsenite spraying, are destined to be lost when shaded by the closing *Hevea* canopy. A thick legume cover suppresses those forest species

* This paper was presented at the Ninth Pacific Scie nce Congress, Bangkok, 1957.

which might otherwise become established. Thus when the legumes are shaded out the area is left free for colonisation by shade-tolerant grasses, especially the widespread alien *Axonopus compressus* which appears to be detrimental to *Hevea*.

Moreover selective hand weeding of grasses and other plants, which are either considered harmful members of the natural cover association or found to suppress the development of desired species, has become expensive. Spraying with sodium arsenite and other herbicides to save labour costs or to meet shortages of labour has lately increased. Most chemicals used over a period of time encourage grass regeneration at the expense of legumes and desirable indigenous species. (One or two chemicals with a measure of selective action against grasses have become available, but they are expensive). Mechanical cultivation has been used occasionally and this also encourages grasses and suppresses woody plants.

There are thus grounds for fearing that it will be increasingly difficult to achieve a desirable succession of vegetation under present day conditions. The trends observed in the botanical surveys reviewed here give some justification for this fear.

THE SURVEYS

The plants have been divided arbitarily into the following classes:

a. Sown legumes, such as *Pueraria phaseoloides*, which may also occur naturally.
b. Grasses (Gramineae only, not in the popular sense).
c. Herbs, other than those included under a, b or d.
d. Creepers and climbers, woody and herbaceous, except grasses and sown legumes.
e. Shrubs—erect woody species generally not exceeding 3 to 4 metres in height and thus unlikely to overtop *Hevea* three or more years old.
f. Trees—erect woody species which would exceed 3 to 4 metres in height if allowed to grow freely.

In the tables two figures are given for each entry; the upper figure is the number of species of the class in question which are judged to be frequent, the lower figure is the total number of species.

The first surveys were made by Mr K. N. Kaimal, who used transects and quadrats on different occasions to obtain representative samples of the vegetation, but the basis on which he judged a species to be frequent is not recorded.

On an Estate Near Seremban

Table 1 refers to an estate near Seremban where the vegetation was studied in adjacent plots; in some of these plots the debris had been burnt between felling the forest and planting *Hevea*, and these were compared with the plots where burning had not taken place. Leguminous covers were not established in these plots and grasses were excluded by hand during the first five years, with the exception of *Panicum pilipes* which was encouraged in the burnt plots. The intention was to control the vegetation by slashing every six months to a height of about one metre, but the war intervened and from the sixth year until the tenth this system of upkeep was abandoned. The surveys reported were made three years and twelve years after planting—about two years before and after the war period.

During the early years *Panicum pilipes*, *Nephrolepis biserrata*, the climbers *Passiflora foetida* and *Vitis trifolia*, the shrub *Trema orientalis*, *Glochidion* sp. and various *Macaranga* species predominated in the burnt plots. Twelve years after clearing, the erect woody species mentioned above had disappeared and there were many patches where the vegetation was sparse. *Panicum pilipes* was still abundant; wild gingers had appeared in quantity, especially *Elettariopsis triloba*, and *Nephrolepis* was quite frequent. An alien shrub *Clidemia hirta* and self-sown seedlings of *Hevea* were the locally dominant erect species.

Throughout the unburnt areas no species was abundant or dominant during the early

TABLE 1. NUMBERS OF SPECIES FOUND IN BOTANICAL SURVEYS ON AN ESTATE NEAR SEREMBAN

Year of clearing	1936			
Nature of clearing	Debris burnt		Debris unburnt	
Year of survey	1939	1948	1939	1948
Column number	1	2	3	4
(a) Sown legumes	0/0	0/0	0/0	0/0
(b) Grasses	1/1	2/2	0/0	0/1
(c) Herbs, other than (a), (b) or (d)	2/3	4/15	2/5	0/11
(d) Creepers and climbers	12/17	3/17	18/22	13/31
(e) Shrubs	5/7	2/15	3/10	0/20
(f) Trees	9/19	1/16	10/20	1/21
Total species	29/47	12/65	33/57	14/84

Upper figure — Number of species judged to be frequent.
Lower figure — Total number of species in class.

years, except perhaps locally a climber *Tinomiscium* sp., although *Nephrolepis*, *Passiflora*, *Vitis* and *Smilax* species were fairly common and *Macaranga* trees frequent.

Twelve years after clearing there was a dense undergrowth beneath the *Hevea* trees in the unburnt plots. Except for *Hevea* seedlings, none of the many shrub and tree species was individually frequent, but climbing plants were very frequent, especially *Agelaea vestita*, *Desmos dasymaschala*, *Limacia velutina* and *Smythea macrocarpa*.

The main contrasts brought out by this summary and Table 1 are that the flora has been poorer and, in later years, the vegetation sparser in the burnt areas than in the unburnt. The stumps of trees, seeds, spores and vegetative propagules in the soil were largely destroyed during burning and such areas were open to colonisation by invading plants. In general, a few species became virtually dominant in the burnt areas, whereas in the unburnt areas there was a strong regeneration of many species, none of which predominated.

Mr K. N. Kaimal reported similar trends in other experiments; unfortunately, detailed data and often the experiments themselves did not survive the war. *Nephrolepis* and *Trema* spp. were frequent colonisers of burnt areas. Where legumes were sown, *Pueraria phaseoloides* was established best in burnt areas, whereas *Centrosema pubescens* was more successful in unburnt clearings.

At the R.R.I. Experiment Station

In 1955 Mr V. K. Bhaskaran Nair made a survey of the R.R.I. Experiment Station at Sungei Buloh. At each site 100 quadrats of 1 square metre were taken. The sites have been arranged in groups of four which had certain features in common. Except for the first column in Table 2, each of the entries in Tables 2 and 3 represents the results from 400 quadrats. A species has been counted frequent if it occurred in 100 or more of these.

TABLE 2. NUMBERS OF SPECIES FOUND IN BOTANICAL SURVEYS ON THE R.R.I. EXPERIMENT STATION

Year of clearing	1934		1929/37	1935	1936/40
Nature of clearing	lightly burnt		burnt	unburnt	Elephant belt
Year of survey	1938	1955	1955	1955	1955
Column number	1	2	3	4	5
(a) Sown legumes	0 0	0 1	0 3	0 0	0 2
(b) Grasses	2 6	0 5	0 4	0 4	0 7
(c) Herbs, other than (a), (b) or (d)	3 19	1 17	1 12	0 12	0 12
(d) Creepers and climbers other than (a) or (b)	1 21	1 44	0 34	0 45	1 43
(e) Shrubs	2 8	0 31	0 28	0 20	1 24
(f) Trees	6 61	0 47	0 42	0 44	0 55
Total species	14 115	2 145	1 123	0 125	2 143

Upper figure — Number of species judged to be frequent.
Lower figure — Total number of species in class.

The first column of Table 2 gives the results obtained by Mr Kaimal using the belt transect method to survey an area where the lighter debris only was burnt after clearing. The second column gives the results of the 1955 survey (in which quadrats were used) of approximately the same area seventeen years later. The numbers of species found in the two surveys are probably comparable, but the estimates of frequency are not.

In this lightly burned area the first invaders were ephemeral Compositae such as *Ageratum conyzoides*, but *Nephrolepis*, *Trema* spp. and the two grasses *Axonopus compressus* and *Paspalum conjugatum* were also abundant. The grasses were later removed by hand weeding, as it was feared that they would suppress other plants. The vegetation was slashed to about one metre every six months.

After four years, in 1938, the ground flora was dominated by *Nephrolepis* and a wild ginger (*Hornstedtia* sp.). The most common erect woody plant was *Ficus alba*, and several other species typical of secondary forest were frequent.

In 1955 two ferns *Nephrolepis* and *Taenitis blechnoides* were the most common plants, but the flora was rich in species of all types.

The other columns of Table 2 refer to areas of differing ages, in all of which the *Hevea* canopy had closed over for many years. The covers throughout were slashed twice a year. Column 3 is an analysis of fields burnt after clearing, column 4 an analysis of unburnt fields. The areas dealt with in column 5 had a peculiar history. After felling in 1929, a thick growth of secondary forest was encouraged as a protection against intruding elephants. When this defence was no longer necessary, planting rows were cleared and planted with *Hevea*, the vegetation between the rows being cut back. This was undertaken in 1936 and 1940 for different portions of the belt. All these areas are adjacent to forest.

The comparison of the flora of these areas, as indicated in Table 2, does not fully support the contrast between burnt and unburnt areas observed on the estate near Seremban (Table 1). As might be expected, no species has emerged as predominant in the unburnt areas; but it is surprising that the flora is not markedly richer in species. Creeping and climbing species are somewhat less frequent in the heavily burnt areas, and *Taenitis blechnoides* has emerged as abundant. These differences from the observations made on the estate near Seremban may be the result of the isolation of some fields from forest as the Sungei Buloh station expanded. The comparison is also weakened because it does not relate to adjacent plots.

The old elephant belts have a rich flora, although not as rich as in freely developing secondary forest of similar age. *Melastoma malabathricum* (a common shrub of secondary vegetation) and *Nephrolepis* are the two frequent species in the old elephant belts. The presence of *Melastoma* may reflect the repeated disturbance of these areas.

Post-war plantings are treated in Table 3. The clearings referred to in columns 1 and 2 were planted from *lalang* and young secondary forest, which had arisen when these areas were abandoned after sporadic food cultivation during the war. In the newer clearings (column 1), sown *Pueraria* was still frequent, but had been lost in the older clearings (column 2). *Mikania, Panicum trigonum* and *P. pilipes* were abundant throughout, with large quantities of *Paspalum conjugatum* in the younger areas which has been replaced by *Axonopus* where the tree canopy has closed. *Scleria* spp. and *Eupatorium odoratum* were also frequent in the older plantings,

TABLE 3. NUMBERS OF SPECIES FOUND IN BOTANICAL SURVEYS ON THE R.R.I. EXPERIMENT STATION

Year of clearing or replanting	1951/2	1948	1949 to 1953
Type	Clearing from *lalang* and secondary growth after food crops		Replanting
Year of survey	1955	1955	1955
Column number	1	2	3
(a) Sown legumes	1 / 3	0 / 2	1 / 3
(b) Grasses	2 / 11	2 / 11	1 / 6
(c) Herbs, other than (a), (b) or (d)	0 / 15	2 / 16	2 / 14
(d) Creepers and climbers other than (a) or (b)	1 / 6	1 / 29	2 / 6
(e) Shrubs	0 / 9	0 / 16	1 / 5
(f) Trees	0 / 4	0 / 21	0 / 8
Total species	4 / 48	5 / 95	7 / 42

Upper figure — Number of species judged to be frequent.
Lower figure — Total number of species in class.

while the occurrence of *Passiflora foetida* and *Panicum nodosum* just fell short of the standard for inclusion as frequent.

The total numbers of species in these more recently planted areas is low compared with the lightly burnt 1934 clearing (Table 2, column 1), but is similar to that in the burnt 1936 clearing near Seremban. However, the balance of species and types has changed, the differences being even more striking when the original quadrat data are studied in greater detail than is practicable here. These differences may be attributed to the changes in cultivation methods discussed earlier.

The third column of Table 3 deals with replantings, all of which had been clean weeded prior to destruction of the old trees and were somewhat isolated from forest. The flora is thus of interest as an example of what may be expected in many replantings. Replanting was spread over the years 1949 to 1953. Certain experimental treatments delayed the development of the canopy in some portions, so that all areas were fairly open when surveyed in 1955.

The only sown legume which was frequent was *Centrosema pubescens*, which may reflect the lack of burning at replanting. A striking lack of tree species, both in number and frequency, was encountered, the replantings being dominated by an association of creeping plants. In addition to *Centrosema*, the main elements were *Paspalum conjugatum*, *Mikania scandens* and *Passiflora foetida*. The erect plants *Cyperus compressus*, *Eupatorium odoratum* and *Melastoma malabathricum* were also frequent. Thus some of the fears expressed in the introduction are justified.

CONCLUSIONS

These observations show how cultivation practices can influence the flora. On an estate near Seremban, burning after clearing had a long-lasting effect. The nature of previous cultivation — rubber or food crops isolation from forest and current methods of upkeep, has resulted in vegetation with a different floristic balance in the post-war plantings compared with the other areas discussed.

Various problems arise which are now receiving attention:

1. Are these changes general and persistent?
2. If so, are the species which are increasing in abundance less favourable to *Hevea* cultivation?
3. Can unfavourable effects be compensated for economically by manuring or must special covers be established and maintained?
4. What species, especially among those which are cheaply established and persist under shade, are most suitable?

There is one final point of general import. Bad cultivation in the past has led to a biotic climax, for example sheet lalang or *Gleichenia* thicket, which renders replanting or reafforestation difficult. Practices leading to impoverished plant communities, such as those dominated by heavy growth of *Axonopus*, may prove equally harmful. In considering the possible dangers of impoverishing the flora it should be borne in mind that successions of vegetation on rubber estates which promote a rich flora are considered desirable to preserve the value of the soil itself.

ACKNOWLEDGEMENTS

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The Soap Titration Method of Estimating the Particle Size of Hevea Latex

B. C. SEKHAR

An investigation was made into the effects of storage, ammonia concentration and temperature on the values obtained for particle size of latices determined by the soap titration method. The marked influence of temperature on the accuracy of the method is discussed. Particle size variation in clonal latices are discussed in relation to their mechanical stabilities. It is shown that the A.S.T.M. mechanical stability test does not determine the time required for the onset of agglomeration of particles.

Particle size of *Hevea* latices has been measured by a number of independent methods (LANGELAND 1936, LUCAS 1938, 1942, WREN 1941 and COCKBAIN 1952). COCKBAIN (1952) modified the soap titration method used for GRS latices (KLEVENS 1947) and successfully applied it to the measurement of *Hevea* latices. The validity of the measurements he made using this method was subsequently confirmed by VAN DEN TEMPEL (1952) who used electron microscopy on brominated latex particles. Recently SCHOON and VAN DER BIE (1955) reported measurements on the particle size distribution of a number of clonal latices using the same method. The present investigation was made to assess the variation in average particle size among clonal latices with a view to relating this property to other important physical properties of the latex, especially its mechanical stability. The soap titration method was chosen for this investigation, since it is inherently simple and requires no specialised equipment. During the present investigation, it was discovered that particle size measurements taken at different temperatures were significantly different. This paper describes the effect of different temperatures on the values obtained for particle sizes of clonal latices.

METHOD

Details of the modified soap titration method for *Hevea* latices have already been described (COCKBAIN 1952) and no alterations were made in the present investigation. Purified sodium dodecylsulphate supplied by Dr E. G. Cockbain was used. The grade of polyvinyl alcohol found most suitable for creaming was Rhodoviol HS-10; this was used throughout.

RESULTS

Variation in Particle Size

Measurements on freshly prepared concentrates, field and skim latices were first made to confirm the values obtained by COCKBAIN (1952) for old latex samples. The range of values obtained is given in Table 1.

TABLE 1. PARTICLE SIZE MEASUREMENT, DATA FROM MALAYA AND GREAT BRITAIN

Sample	Present data		Cockbain's data	
	Specific surface area (S), $cm^2 \times 10^4$	Volume surface average diameter (D), microns	S $cm^2 \times 10^4$	D microns
Field latex	6 – 14	1.0 – 0.43	18 – 20	0.33 – 0.3
Concentrate	3.5 – 6	1.72 – 1.0	8 – 11	0.75 – 0.5
Skim	30 – 34	0.2 – 0.18	52	0.12

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TABLE 2. EFFECT OF STORAGE ON PARTICLE SIZE

Sample	No storage		3 months' storage	
	Specific surface area (S) $cm^2 \times 10^4$	Volume surface average diameter (D), microns	S $cm^2 \times 10^4$	D microns
Field latex	8.2	0.73	8.5	0.71
Concentrate	5.6	1.07	5.9	1.02

There is a significant difference in values obtained from old preserved latices in Great Britain and those from freshly prepared samples in Malaya. Unless the particle size decreases on storage, which seems unlikely, the cause of such a difference has to be sought elsewhere. To investigate the possibility of storage effects, tests were made on latices which had been kept for over three months in Malaya. These results are shown in Table 2. No significant differences were found.

The effect of ammonia concentration was next investigated. This was carried out by estimating particle size of samples of latex from the same source ammoniated to different concentrations. The results presented in Table 3 show that although ammoniation of latex below 0.5% causes variation in the amount of soap adsorbed, values obtained for samples above 0.5% ammonia concentration were quite consistent.

Since these results indicated no appreciable effect due to storage or ammonia concentration over 0.5%, the next most likely factor, the temperature at which measurements were made, was investigated. The measurements carried out by Cockbain were at a temperature of about 22°C while in Malaya room temperature corresponds to nearly 30°C. Estimations were therefore carried out at these two temperatures, and also in two cases at 50°C, on a number of samples of latex. Results obtained are given in Table 4. It is clear from these results that the values obtained by this method of estimation are markedly dependent on temperature. Table 4 also shows that the comparative difference between latices is maintained at both temperatures within experimental error. The method of estimation is therefore of use for comparative purposes.

Particle Size of Clonal Latices

Samples of clonal latex were collected from two different sources and particle size determinations were carried out. The determinations were made at a temperature of 22°C. Results are summarised in Table 5.

TABLE 3. EFFECT OF AMMONIA CONCENTRATION ON PARTICLE SIZE MEASUREMENT

% ammonia concn in latex (wt/volume)	Specific surface area (S) $cm^2 \times 10^4$	Volume surface average diameter (D), microns
0	3.5	1.70
0.2	4.4	1.36
0.3	4.7	1.28
0.5	4.7	1.28
0.6	5.2	1.15
0.7	5.2	1.15
0.9	5.2	1.15

TABLE 4. EFFECT OF TEMPERATURE ON PARTICLE SIZE MEASUREMENT

Sample	Specific surface area (S), $cm^2 \times 10^4$		
	22°C	30°C	50°C
Mixed seedling field latex	18.7	9.1	7.2
Mixed seedling latex concentrate	9.0	6.0	4.5
Gl 1 field	24.7	12.8	—
Gl 1 concentrate	16.0	9.3	—
AVROS 50, field	18.1	9.1	—
AVROS 50, concentrate	12.0	6.5	—
PB 186, field	15.0	7.4	—
PB 186, concentrate	10.0	6.0	—

TABLE 5. VARIATION IN PARTICLE SIZE OF CLONAL LATICES

Clone	Source 1		Source 2	
	Specific surface area (S) $cm^2 \times 10^4$	Volume surface average diameter (D), microns	S $cm^2 \times 10^4$	D microns
Gl 1	16.0	0.38	18.9	0.32
Tjir 1	11.3	0.53	13.6	0.44
RRIM 501	15.0	0.40	15.0	0.40
PB 186	11.3	0.53	13.4	0.45
PB 86	13.4	0.45	15.1	0.40
BD 5	17.1	0.35	18.5	0.34
Pil B84	11.2	0.54	15.4	0.39
RRIM 519	13.8	0.43	20.0	0.30
PB 23	13.6	0.44	—	—
Pil A44	13.6	0.44	—	—
AVROS 49	11.1	0.54	—	—

The variation in particle size between clones is not quite consistent in latices collected from two sources as is evident from the Table; thus in some cases variation due to the source of collection may offset any variations due to clonal characteristics. This conclusion is also supported by the observation of COCKBAIN (1952) on the particle size of the mechanically unstable Gl 1 latex. Cockbain observed that Gl 1 latex generally contains particles of larger size than do other clonal latices, an observation contrary to the results reported in this paper.

Effect of Particle Size on Mechanical Stability

Samples of latex from different clones were collected and particle size determinations on

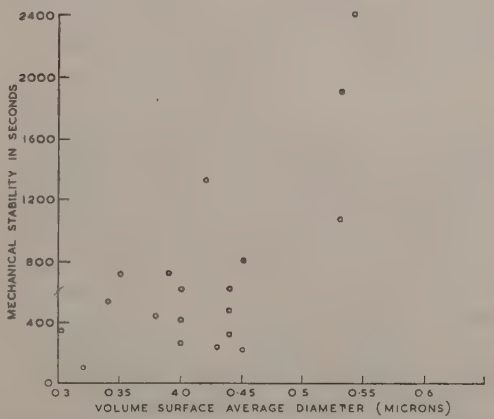

Figure 1. Effect of particle size of clonal field latex on mechanical stability.

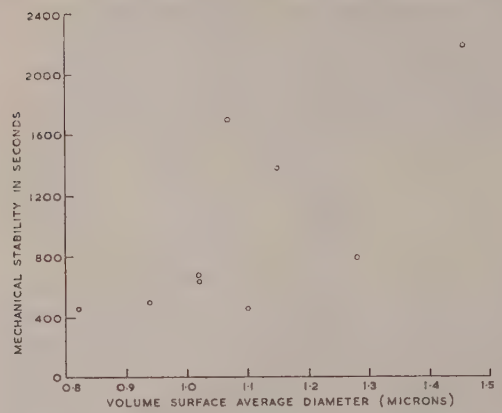

Figure 2. Effect of particle size of clonal latex concentrate on mechanical stability.

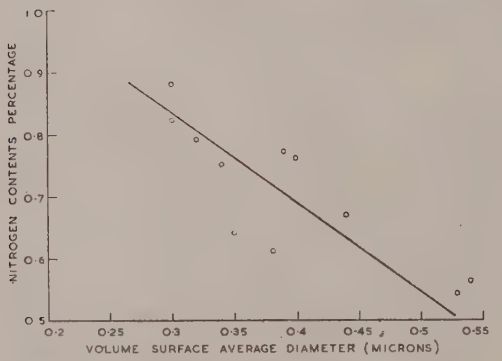

Figure 3. Effect of nitrogen contents of field latex on particle size.

both the field latices and centrifuged concentrates were carried out at 22°C. The mechanical stabilities of these concentrates were determined at the end of 30 days storage. Nitrogen estimations were also carried out on the total solids films of the field latices. All these values are summarised here in graphs. Mechanical stability is plotted against particle size of clonal field latex (Figure 1) and against particle size of clonal concentrate (Figure 2). In Figure 3 the nitrogen content of field latex is plotted against particle size.

It is evident from Figures 1 and 2 that mechanical stability is not simply related to the particle size of the field or concentrate latices. Figure 3 indicates that the particle size and nitrogen contents of the latices are related.

Effect of Wintering on Particle Size of Clonal Latices

To investigate the variation in particle size of clonal latices during and after the wintering period, a number of measurements were carried out on a few latices during the months of October, December, March, April and July. Clonal latices from the same field at the R.R.I. Experiment Station were collected each time. Values obtained are summarised in Table 6.

TABLE 6. PARTICLE SIZE VARIATIONS DURING WINTERING

Month	Specific surface area (S), $cm^2 \times 10^4$			
	Gl 1	PB 186	BD 5	RRIM 501
Oct.	19.8	15.4	—	—
Dec.	16.0	11.3	17.0	15.0
* March	12.6	8.9	13.8	12.4
* April	10.8	12.2	11.0	10.4
July	14.0	15.1	14.8	13.0

* Wintering months

Thus the latices show a definite trend towards an increase of particle size during wintering. Both the rate and extent of increase are different for different clonal latices and these increases may also depend on the source of the latex.

Effect of High Speed Mechanical Agitation on Particle Size of Latex

It is of interest to determine whether the agglomeration of latex particles taking place on high speed mechanical agitation is a slow gradual process or a sudden one. This investigation was carried out by stopping the Klaxon stirrer (14,000 r.p.m.) during an A.S.T.M. mechanical stability test after varying periods of stirring and determining the particle size of the resulting samples. It was found possible to

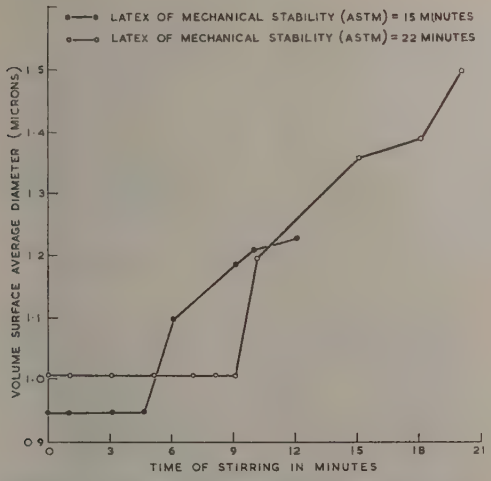

Figure 4. Effect of mechanical agitation on particle size.

determine the particle size only for a definite period of stirring with each latex. After this period coagulation occurs while acid is being added to reduce pH. Figure 4 gives the typical results obtained. For a certain period of agitation no change in particle size occurs; then there is a sudden increase followed by a gradual change. In a latex that had a mechanical stability of 22 minutes, the sudden increase took place after 9 minutes of stirring after which the change in particle size became gradual until, at 20 minutes stirring, the latex became too unstable for further measurements to be made.

DISCUSSION AND CONCLUSIONS

Tables 1, 2 and 4 clearly demonstrate that the soap titration method of assessing particle size is dependent on temperature. A number of reasons may be advanced for this phenomenon, among which the most likely are:

1. The extent of adsorption of soap increases with decrease in temperature, since at high temperature the latex particles are not completely covered with soap and the extent of coverage increases with decrease in temperature.
2. At lower temperatures multiple layers of soap are formed at the surface instead of a monomolecular layer.
3. The molecular adsorption area of the soap increases with increase in temperature; in other words, by expansion at higher temperatures the same area of soap film is sufficient to cover a larger surface.
4. Cluster formation of latex particles is suggested by SCHOON and VAN DER BIE (1955). If latex particles form clusters, then it is conceivable that they are affected by temperature, which would mean that higher temperatures would facilitate cluster formation. Further work would have to be carried out to elucidate this phenomenon.

The results obtained at two temperatures, 22 and 30°C, are consistent in regard to differences between clones. The electron microscope data (VAN DEN TEMPEL 1952) reported in the literature favour the values obtained at 22°C. However this in itself does not constitute proof that the measurement at 22°C provides absolute values, since only values for distribution of particle size can be obtained with the electron microscope and average size values cannot be estimated with any accuracy.

Results presented in Tables 5 and 6 indicate that the variation within any one clonal latex due to source and month of collection could be as great as the variation between clones. It is doubtful, therefore, whether clonal characteristics determine particle size of latex to any significant extent.

There is no direct relationship between mechanical stability of latex and apparent particle size, as can be seen from Figures 1 and 2 in which mechanical stability values are plotted against particle size. There is however an indication that unstable clonal latices contain smaller sized particles. The absence of any direct relationship is understandable, since it is known that several factors influence mechanical stability, among which P/Mg ratio (PHILPOTT and WESTGARTH 1953), aeration (COLLIER 1955) and V.F.A. No. (COOK and SEKAR 1955) are important ones.

Figure 3 indicates that there is a significant relationship between nitrogen content and particle size. Since most of the nitrogen is derived from proteins which themselves are adsorbed at the particle/serum interface, one would expect such a relationship to exist.

High speed mechanical agitation does not cause any immediate agglomeration of latex particles. It is only after an appreciable period of stirring that a sudden 15–20% increase in size of particles takes place and thereafter the increase is gradual. These facts are evident from Figure 4. It is also clear from Figure 4 that the A.S.T.M. mechanical stability test is not a measure of the onset of agglomeration of particles, but is probably a measure of the approximate time taken to attain a definite particle size visible to the naked eye.

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Seed Germination of some Tropical Legumes

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An account is given of the influence of seed treatment on the germination of Calopogonium mucunoides, Centrosema pubescens, Flemingia congesta and Pueraria phaseoloides. Among the treatments studied are: storage conditions, mechanical scarification, concentrated sulphuric acid treatment, and immersion in water, glycerine and kerosene. A brief report is also given of the effects on germination of Gibrel, Kinetin and infra-red irradiation.*

Three creeping legumes, *Calopogonium mucunoides* Desv., *Centrosema pubescens* Benth. and *Pueraria phaseoloides* Benth., and the leguminous shrub *Flemingia congesta* Roxb., are extensively used as cover crops and green manures in Malayan agriculture. There is some doubt if the use of legumes for these purposes is beneficial in all circumstances. There are, however, cases where the establishment of a leguminous cover crop is the only practical solution to the problems of erosion control and the exclusion of undesirable weeds. When sown legumes are employed, establishment should be as rapid as possible in order to cover the soil before erosion can take place and before weeds can invade or regenerate.

If untreated seed is used the percentage germination is often low and establishment is uneven and slow. It has consequently become a common practice to treat leguminous cover plant seeds in one way or another with a view to improving germination. This paper is a report on experiments in which we have tested the effect on germination and establishment of various seed treatments, including conditions of storage.

CHEMICAL & MECHANICAL SCARIFICATION; LIQUID IMMERSION; STORAGE CONDITIONS

Current Practices: Sulphuric Acid and Hot Water Treatments

In Figure 1, commercial samples of seed are grouped by percentage germination with and without pretreatment (by chemical scarification) with concentrated sulphuric acid followed by washing. Pretreatment gives higher percentage germination. The difference between the germination in the two tests divided by the germination after acid treatment defines the 'hard seed fraction' or proportion of seed which will not germinate within 21 days without effective pretreatment. The hard seed fraction varies considerably (Figure 1).

In Figure 2 the cumulative germination over 21 days is given for untreated and acid treated seeds. Effective treatment increases both the percentage germination and the rate of emergence. These are beneficial effects in field practice in that seeding rates may be reduced, adequate numbers of seed will germinate even if the hard seed fraction is high, and the period of establishment is reduced.

Two methods have been used in field practice hitherto: (i) sulphuric acid pretreatment (ii) immersion in warm or hot water. In the latter method the seeds are immersed in hot water at 50°C for periods up to 16 hours or immersed in water at 70°C and left to cool. Application of the acid treatment on a large scale involves some danger to the operatives and protracted treatment can damage the interior of the seed. The hot water treatment is not always effective. For instance, the supposedly optimal hot water treatment for each species as determined in earlier work effected

* This paper was read by the author at the Ninth International Botanical Congress, Montreal, 1959.

no improvement in: 19 out of 86 samples of *Calopogonium*, 25 out of 113 samples of *Centrosema*, 8 out of 14 samples of *Flemingia*, 11 out of 156 samples of *Pueraria*.

The current hot-water methods have a further disadvantage. The seeds are washed or soaked in water and unless they are rapidly dried after treatment before germination commences, the absorbed water allows germination to take place in the field even if there is inadequate moisture in the soil. Complete loss may result if rain fails after sowing. The object of these studies is to seek safe and simple methods of treating seed which will enhance the percentage germination and the rate of emergence without increasing drought susceptibility.

Effect of Storage Conditions

The testa of a fully ripened seed is impervious to water and if the path of water entry is closed the seed becomes hard. The results of the storage experiment reported in Figure 3 indicate that damp conditions increase the proportion of hard seed. Seeds were stored in a well ventilated dry room. Humidity fluctuated from 50% by day to nearly 100% at night; the temperature range was 25°C to 32°C, but usually 30°C by day and 28°C by night. These conditions are referred to hereafter as normal room conditions. Seeds of the same origin were stored in a cabinet under the same temperature conditions but in a water saturated atmosphere.

For the storage experiment, only 'good seed to the eye' were used. At monthly intervals a sample of 600 seeds of each species was withdrawn from each set of conditions and after any seed which no longer appeared good had been removed the sample was divided into halves. One half of the sample was tested for germination without further treatment and the other half set out for germination after the testa had been cut with a scalpel. This 'cutting' treatment is very effective and gives results comparable with optimum acid treatment. In all cases the germination with or without cutting treatment declined during storage. In particular, there was a marked reduction in the germination of seed which received no cutting treatment after storage under damp conditions, so that the hard seed fraction rose as a result. This is in agreement with the report (HYDE 1954) that the hard seed fraction increased in other leguminous seeds stored at a constant high humidity.

Immersion in Warm Liquids

A saturated atmosphere during storage has a depressing effect on germination without pretreatment, and immersion in hot water is not always effective as a pretreatment. Therefore immersion in other liquids was tried. Figure 4 shows the results of immersion in glycerine, water and kerosene (commercial paraffin fuel) at room temperature (28–30°C) and at 50°C. For the latter the liquids were raised to 50°C before the seeds were introduced and held at that temperature for the periods indicated. In these tests the glycerine was not washed off but was allowed to adhere to the seed; neither was it possible to remove the kerosene completely—only the excess was cleaned off. The controls were cut and uncut seed not subjected to immersion treatment.

In this and all other experiments the seeds were set out for germination on moist Whatman seed-test papers (1/16 in. thick, 9.0 cm diameter) in covered, unglazed porcelain blocks, 100 seeds per block. Germinated seeds were counted and removed daily and the papers kept moist. Emergence of the radicle was the criterion of germination. Tests ran for 21 days.

A summary of the results follows.

1. *Calopogonium.* Glycerine was effective in promoting germination, especially warm glycerine. The longer periods of immersion in warm kerosene were depressive.
2. *Centrosema.* Glycerine was effective, especially warm. Warm water and kerosene promoted germination to some extent.
3. *Flemingia.* All warm liquids were effective. Cool kerosene was effective at 40 minutes immersion.

4. *Pueraria.* All warm liquids were effective, especially glycerine and kerosene. Cool glycerine was only slightly effective and cool kerosene was markedly effective during short periods of immersion.

In general, cool water was ineffective and warm water gave variable results. Cool glycerine was effective in certain species and warm glycerine was effective in all cases. Kerosene gave variable results, but was more effective when warm.

A further experiment was undertaken in which the seeds were immersed in kerosene and glycerine at different temperatures and left to cool for two hours. The results are given in Figure 5. Apart from a decline in most cases at the higher temperatures employed and a tendency for glycerine to be more effective than kerosene, the results may be summarised as follows:

1. *Calopogonium.* Glycerine effective.
2. *Centrosema.* Kerosene effective and glycerine very effective.
3. *Flemingia.* Kerosene and glycerine fairly effective but variable.
4. *Pueraria.* Glycerine and kerosene very effective.

Mechanical Scarification and Dry Warmth

The effects of two other treatments are illustrated in Figure 6: (i) mechanical scarification by rotation (75 r.p.m.) in a hexagonal drum, the interior lined with sand paper and (ii) the effect of dry warmth. The body of each diagram shows the effects of these treatments for various periods on one sample of seed of each species. The left hand column (B.S.) shows the results of 28 hours mechanical scarification of seed in bulk from another source for field trial. In all cases scarification for 28 hours promoted germination. Scarification for only 4 or 8 hours improved germination in *Flemingia* and *Pueraria.* Dry warmth gave variable results: it was depressive in *Flemingia,* ineffective in *Calopogonium,* just effective over a period of 2 to 8 hours in *Centrosema* and very effective in *Pueraria.*

Collation of Results

Thus far we find that treatments which cut, or chemically or mechanically scarify the testa are effective. It should be noted that scarification with sulphuric acid is also a heat treatment owing to the heat generated locally when the seeds are immersed in the acid and during the washing away of the acid with water. Dry warmth has a variable effect. Generalising, immersion in warm liquids is more effective than immersion in cool liquids and these non-acid liquids are effective in the order: water least, kerosene intermediate and variable, glycerine most effective. The pure glycerine used in these experiments has a strong osmotic concentration when in contact with aqueous systems and may owe its effectiveness to the extraction of water, which is consistent with the fact that humidity during storage depresses germination. Solution of impermeable substances by glycerine and kerosene may be a contributory factor where these liquids are effective. However, a preliminary trial with the fat solvent ethyl alcohol was not promising.

Effects of Treatments on Viability

The question arose whether the less effective treatments merely failed to promote germination or whether beneficial effects were counterbalanced by a reduction in the viability of the seed. Therefore seeds were subjected to various pretreatments discussed in the previous experiments for whatever period appeared to be optimal in those experiments. Afterwards they were set out for germination (i) without further treatment and (ii) after cutting the seed coat. The results are given in Figure 7. Despite a few anomalies (e.g. where cutting after treatment gave lower germination than treatment alone), the overall effect of pretreatments was on germination itself without affecting to any marked degree the total proportion of seed capable of germinating.

Miscellaneous Observations

Other experiments, not reported in detail here, allow us to draw the following conclusions.

Chilling seed, wet or dry for 1 to 7 days at 1 to 5°C, had a depressing effect on germination.

Immersion in a mixture of 50% glycerine and 50% water by volume had virtually the same effect as water whether cool or warm. However, a preliminary trial showed crude glycerine from the soap factory (approx. 80 – 82% glycerine, 8% common salt, remainder water and other residues) to be of equivalent effect, when used warm, to pure glycerine: thus the physical properties (osmotic concentration and temperature) of the solution may be important.

During storage, especially under damp conditions, the total viability (i.e. proportion of total seed which will germinate after cutting) declined more rapidly in scarified and warm-glycerine treated seed than in untreated seed. This deterioration was greatest for glycerine treated seeds. However, scarification and warm glycerine treatment decreased the proportion of hard seed and this fraction remained almost constant for seed so treated during storage under normal or damp conditions. Thus, despite the general decline in percentage germination, seed do not apparently become 'hard' again after these treatments. It is advisable to use seed soon after treatment, although scarified seed was stored for two months and glycerine-treated seed for one month without severe deterioration.

The total viability of untreated seed was maintained without increase in hard seed fraction during a storage period of over eighteen months in a dehumidified room (relative humidity 50 – 55%).

No differences have been detected between germination in covered, unglazed, porcelain blocks and in glass petri dishes, i.e. exposing the germinating seed to daylight does not appear to influence germination. Germination proceeds at the same rate in an air conditioned room in which the temperature is reduced to 23°C for 8 hours each day as in a room where normal conditions prevail, i.e. within these limits ambient temperature during germination has little effect.

Pot and Field Trial of Promising Methods

Table 1 shows results for a pot experiment on the effects of pretreatments on germination, growth and nodulation. The experimental design was factorial without replications: 4 species × 4 pretreatments × 8 combinations of seed dressings. Only pretreatment effects are considered here. Plants were harvested three months after sowing.

The results of a field trial to study the effect of pretreatments on the establishment of the legumes are shown in Table 2. A replicated factorial design was used: 4 species × 4 pretreatments × 16 combinations of seed dressings × 4 replications. Only pretreatment effects are considered. Each plot was a 38 ft length of drill, 4 ft from either adjoining drill. Observation was at three months after planting and a score of 0 to 5 was given for general appearance. The number of plants in the central foot of each drill was counted and their spread outwards from the centre of the drill was measured in inches.

The optimum treatments for each species determined in previous experiments were applied. The warm kerosene treatment was included in the pot experiment because, although this was not the most promising of treatments, it was much less expensive than glycerine. In the future the use of the cheaper crude glycerine may provide a solution to the cost problem. The greater weights of plants and nodules obtained after acid and warm glycerine treatment may be attributed largely to accelerated emergence, although there were various interactions. For instance all pretreatments lead to an increased weight of plants and nodules in *Pueraria* and the converse in *Flemingia*. The field trial confirms the value of warm glycerine and scarification methods for establishing the creepers and of scarification for *Flemingia*.

The warm glycerine and sulphuric acid treatments are effective on certain species which do not respond to mechanical scarification, for instance *Stylosanthes guyanensis* (Aubl.) Sw., whose seed is protected by the fruit pod, and *Clitoria rubiginosa* Juss. ex

Pers. whose seed is sticky. The inconvenience of the glycerine adhering to seeds is overcome and turned to practical value by mixing in ground rock phosphate with the seed to absorb the glycerine. The result is excellent placement of this most essential fertiliser for the establishment of legumes on Malayan soils.

GIBREL AND KINETIN

Recently there has been widespread interest in the properties of gibberellins and 6-(substituted) purine compounds. For instance SKINNER *et al.* (1958) have reported on the effects of these substances on lettuce, grass and clover seed germination. The results of an experiment using Gibrel are given in Table 3. Untreated seed and cut seed were set out for germination on the Whatman seed-testing papers soaked in 15 ml of solutions of appropriate strength. There were 100 seeds in each covered, unglazed porcelain block, and five blocks per treatment. The seeds were watered daily to maintain moist conditions, but the solutions were not washed away.

The results show no improvements of practical importance by Gibrel treatment. The improvement due to cutting was highly significant throughout. Only in the case of *Flemingia* seed which had already been cut was there any consistent, significant enhancement of germination by Gibrel treatment; some improvement in germination of uncut *Flemingia* seed was also obtained but it was not significant. In *Calopogonium*, 10 p.p.m. Gibrel depressed the germination of cut seed, probably significantly ($P = 0.05$), although this concentration gave the highest germination of uncut seed; the reverse results were obtained in *Centrosema* and *Pueraria*. Plotting the cumulative germination daily gave no evidence of accelerated or retarded rate of germination due to Gibrel. Cutting accelerated germination in all cases.

The results of a further test to see whether there is any synergistic effect of Gibrel and Kinetin on otherwise untreated seed are given in Table 4. Three seed germination blocks with Whatman seed-test papers wetted with 15 ml of solutions of appropriate strength were used for each treatment, 100 seeds per block. After setting the seed, the moisture was maintained by watering daily, but the solutions were not washed away. Cut seeds, not treated with Gibrel or Kinetin, were provided as a control to show the potential germination. Only in the case of *Centrosema* were the responses to 10 p.p.m. Gibrel and 10 p.p.m. Kinetin probably significant, but these small improvements were not of practical importance. No effect of Gibrel or Kinetin on the rate of germination could be detected.

INFRA-RED LAMP IRRADIATION

Finally, the effects of irradiation with infra-red lamps were tested. The lamps available were Philips Infraphil Type 13373F/479 (150 watts) and Osram IRR 4892 (250 watts). The lamps were suspended about 65 cm vertically above the seed, which were in open dishes near the centre of the light cone. The light was not filtered. The Philips lamp gives a relatively narrow cone of red and infra-red light; the air temperature below the lamp is about 36°C but thermometers in the position of the seeds record temperatures up to 50°C. The Osram lamp gives a wide cone of illumination, which appears to be a white light but extends into the infra-red; the ambient temperature below the lamp is about 35°C and thermometers in the position of the seeds record temperatures up to 43°C. These figures refer to stable conditions after the lamps have been burning for some hours.

According to information supplied by the makers, the total radiant energy at the position of the seeds is about 60 milliwatts per square centimetre and 35 to 45 mW/cm^2 under the Philips and Osram lamps respectively. Certain wavebands have been found to affect germination in other species. These are blue (about 0.45 microns—slightly inhibitory), red (about 0.67 microns—stimulatory) and near infra-red (about 0.73 microns—inhibitory); the Osram lamp transmits relatively more visible light than the Philips lamp. For instance, the latter does not emit any blue at

all. However, the Philips radiates relatively slightly more of the heating infra-red and under the conditions of the experiment much more in absolute units than the Osram—as the details given above indicate.

After the attainment of stable conditions, seeds were exposed to the lamps separately for periods from 7½ minutes to 64 hours and afterwards transferred to germination blocks. The results are given in Figure 8. In the three creeping species there was a tendency for the shorter periods of irradiation to depress germination, and this depression reached significance ($P = 0.01$) at certain points for *Calopogonium* and *Pueraria*. However, longer periods of irradiation reversed this effect. Significantly improved germination was obtained by 8 hours Philips irradiation of *Calopogonium*, by 16 or more hours Osram irradiation of *Calopogonium* and *Centrosema*, by 2 hours with Osram on *Pueraria* and 1 hour or more with Philips on *Pueraria*. Possibly certain wavebands of light had a depressing effect which was countered by the warming effect of longer exposure in these species; dry warmth promoted germination in *Centrosema* and did so markedly in *Pueraria*.

Borthwick *et al.* (1952) have reported promotion of lettuce seed germination by red light and inhibition by infra-red: such processes may be involved here, but the wide spectra from our unfiltered lamps preclude further inferences at present.

The irradiation of *Flemingia* seed for the shortest period used (7½ minutes) resulted in a marked improvement in germination which was confirmed for the Philips lamp in a second test (upper graph), in which the germination was even better than that of cut seed in certain instances. Compared with the other species the contrast was striking, especially because *Flemingia* seed germination was depressed by dry warmth, although usually enhanced by immersion in warm liquids. The only other instances where there has been evidence of germination superior to that of cut but otherwise untreated seeds were for cut seeds of *Centrosema* treated with 10 p.p.m. Gibrel and for cut seeds of *Flemingia* treated with 1, 10 and 100 p.p.m. Gibrel. It may be premature to draw conclusions at this stage, but the behaviour of *Flemingia* seed differs in several ways from that of the other species studied. Although *Flemingia* conforms with them in that impermeability appears to be the main factor involved in 'hardness' of seed, there are traces of other physiological processes.

DISCUSSION

Mechanical and chemical scarification of leguminous seeds to improve germination are long established practices. Sulphuric acid scarification of lupine seeds has been shown (Burns 1959) to erode pits through the testa, or to hydrolyse the counter palisades of the hilum so that they can no longer close the hilar fissure when there is moisture outside the seed in excess of that within. The external humidity and internal moisture content of leguminous seeds have been investigated (Hyde 1954) in relationship to opening and closing of the hilar fissure. The improvement of germination by scarification and the increase in hard seed fraction during storage in constant damp conditions indicate that similar processes are probably involved in the four species studied here, namely the impermeability of the testa in fully ripened seed and the control of the normal path of water entry by a hydrostatic mechanism. Improvements in germination by immersion in glycerine can be explained by such hydrostatic considerations.

Dry warmth promoted germination in *Centrosema* and *Pueraria* and heat enhanced the effects of liquids in all species. Apart from the anomalous depression of germination by dry warmth in *Flemingia*, warmth in some form appears to be beneficial in all species. The effect of warmth may be by action on a bi-layered mechanism such as the hilar palisade.

Recently, interest has centred upon such substances as gibberellins and 6-(substituted) purines, for example Kinetin. Gray (1957) reported accelerated germination of beans and peas by application of gibberellin slurries.

Skinner *et al.* (1958) found increased germination in white clover in response to Kinetin but not to Gibrel. We have detected only relatively minor responses to either Gibrel or Kinetin in the four tropical species studied.

In that wide spectra were permitted, our irradiation of seeds by infra-red lamps has not been critical. An improvement in germination was effected by long periods of irradiation in the creeping species, which may be explained by heat adsorption. There was also evidence in these species of depressing effects after short periods of irradiation, but similar short periods stimuated an improvement in the germination of *Flemingia*. These experiments indicate that infra-red (or other radiations) may have physiological effects on seed of these species.

A number of anomalies arose. For example, immersion in glycerine for two hours at room temperature, approx. 30°C by day, (Figure 4) was really the same treatment as immersion in glycerine at 30°C for two hours (Figure 5), yet the latter would appear to be much more effective in promoting germination of *Centrosema* seed. In the experiment illustrated in Figure 7, immersion of *Flemingia* and *Pueraria* seed in cold kerosene for 40 minutes failed to reproduce the enhancement found in the experiment to which Figure 4 relates; moreover, scarification was not effective on *Pueraria* in this instance. In view of the variability of hot water treatment mentioned in the introduction, especially with respect to *Flemingia*, the good response of this species in the experiments (Figure 4 and 7) is perhaps surprising. The results in Table 3 show that 10 p.p.m. Gibrel probably depressed germination of uncut *Centrosema* and *Pueraria* seed significantly, yet there is no evidence of this in Table 4 but rather the converse. Neither do the tests of infra-red lamp irradiation on *Flemingia* seed agree completely. Nevertheless, most of these effects mentioned could be established as significant in each individual experiment.

In each individual experiment every effort was made to use uniform 'good' seed from one source. However, the anomalies noted above, the variability in response of commercial samples to hot water treatment and the variability in hard seed fraction itself must serve as warnings that no two populations of seed necessarily consists of similar proportions of seeds in the same physiological condition. At present we have no inbred lines or horticultural varieties in these species which might be less variable than the present supply, nor can we guarantee that all samples of seed have the same history. We must treat these investigations as exploratory at this stage.

SUMMARY

1. Storage under constant damp conditions depresses the total viability and increases the hard seed fraction in *Calopogonium mucunoides, Centrosema pubescens, Flemingia congesta* and *Pueraria phaseoloides.*

2. Scarification, mechanically, by cutting and by concentrated sulphuric acid, promotes germination in all four species.

3. Dry warmth promotes germination in *Pueraria* and to a lesser extent in *Centrosema.* Immersion in warm liquids, especially glycerine, promotes germination. Cool glycerine improves germination to some degree in many cases.

4. The above phenomena are explicable on the assumption that a hard seed has an impermeable testa and that water enters by a path which is hydrostatically controlled.

5. Treatments (2) and (3), above, do not seem to have any marked effect on the viability of the seed but only to effect its germination, in contrast to damp storage. See (1) above.

6. Only minor responses to Gibrel and Kinetin have been detected.

7. Irradiation with unfiltered infra-red lamps suggests that some radiations may depress germination in three species, but this process is reversed by longer periods of irradiation, probably owing to heating effects. *Flemingia* showed a marked response to comparatively brief irradiation.

8. In their responses to damp storage, scarification and immersion in cool or warm liquids, there is considerable agreement between the four species. However, *Flemingia congesta* differs to a certain extent from the other species in its response to dry warmth, Gibrel and infra-red lamp irradiation.

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TABLE 1. EFFECT OF SEED PRETREATMENT ON GERMINATION, PLANT SIZE AND NODULATION. POT EXPERIMENT

Pretreatment	Percentage germination					Dry wt* whole plants, grams					Dry wt* nodules, grams				
	C.m.	C.p.	F.c.	P.p.	Means	C.m.	C.p.	F.c.	P.p.	Means	C.m.	C.p.	F.c.	P.p.	Means
Untreated	25.4	54.7	75.5	39.0	48.7	59.9	88.1	40.5	55.9	61.1	1.89	4.31	1.07	3.56	2.71
Acid treated	23.5	82.3	54.5	82.1	60.6	77.7	98.7	37.9	81.9	74.1	1.84	4.90	0.81	7.01	3.64
Hot glycerine	35.0	80.3	74.9	75.4	66.4	80.1	89.8	35.4	78.5	71.0	1.87	4.59	0.92	4.34	2.93
Hot kerosene	17.1	50.8	80.8	49.8	49.6	63.9	64.7	31.8	61.9	55.6	1.56	3.86	0.80	4.44	2.66
Means	25.3	67.0	71.4	61.6		70.4	85.3	36.4	69.6		1.79	4.41	0.90	4.83	

* Weights refer to 10 plants

s.e. any pair of interaction means	± 3.05	± 4.30	± 0.177
s.e. any pair of main effect means	± 1.53	± 2.15	± 0.089
min. 5% sig. diff. any pair of interaction means	8.6	12.1	0.50
min. 5% sig. diff. any pair of main effect means	4.3	6.1	0.25

C.m. *Calopogonium mucunoides* C.p. *Centrosema pubescens* F.c. *Flemingia congesta* P.p. *Pueraria phaseoloides*

TABLE 2. EFFECT OF SEED TREATMENT ON GENERAL APPEARANCE, ESTABLISHMENT AND SPREAD OF COVER PLANTS, THREE MONTHS AFTER SOWING. FIELD EXPERIMENT

Pretreatment	Score for appearance					No. of plants per foot					Spread in inches				
	C.m.	C.p.	F.c.	P.p.	Means	C.m.	C.p.	F.c.	P.p.	Means	C.m.	C.p.	F.c.	P.p.	Means
Untreated	2.9	2.8	2.9	3.5	3.0	3.6	2.9	6.8	6.0	4.8	12.9	11.6	5.8	11.9	10.6
Acid treated	3.6	3.2	2.3	3.4	3.1	3.9	3.0	2.7	4.4	3.5	21.2	19.2	5.0	15.9	15.3
Hot glycerine	3.6	3.6	3.0	3.8	3.5	4.3	4.2	5.7	6.5	5.2	21.3	15.6	6.4	16.5	15.0
Scarified	3.4	3.3	3.1	3.4	3.3	4.0	4.1	7.5	6.3	5.5	18.0	13.3	6.8	17.2	13.8
Means	3.4	3.2	2.8	3.5		4.0	3.6	5.7	5.8		18.3	15.0	6.0	15.4	

s.e. any pair of interaction means	± 0.135	± 0.395	± 1.504
s.e. any pair of main effect means	± 0.068	± 0.198	± 0.752
min. 5% sig. diff. any pair of interaction means	0.4	1.1	4.2
min. 5% sig. diff. any pair of main effect means	0.2	0.5	2.1

C.m. *Calopogonium mucunoides* C.p. *Centrosema pubescens* F.c. *Flemingia congesta* P.p. *Pueraria phaseoloides*

TABLE 3. EFFECT OF GIBREL SOLUTIONS AND CUTTING ON GERMINATION. 5 × 100 SEEDS PER TREATMENT

Cutting treatment	(a) *Calopogonium mucunoides*									
	Percentage germination					Transformed to degrees				
	Gibrel p.p.m.					Gibrel p.p.m.				
	0	1	10	100	Means	0	1	10	100	Means
Uncut	39.2	41.6	44.2	37.2	40.6	38.76	40.16	41.65	37.56	39.53
Cut	96.4	96.0	94.0	94.4	95.2	79.27	78.53	75.94	76.56	77.58
Means	67.8	68.8.	69.1	65.8	—	59.01	59.34	58.80	57.06	—

Cutting treatment	(b) *Centrosema pubescens*									
	Percentage germination					Transformed to degrees				
	Gibrel p.p.m.					Gibrel p.p.m.				
	0	1	10	100	Means	0	1	10	100	Means
Uncut	35.2	36.2	28.0	37.0	34.1	36.35	36.98	31.91	37.45	35.67
Cut	79.8	84.4	87.2	83.4	83.7	63.45	67.32	69.22	66.01	66.50
Means	57.5	60.3	57.6	60.2	—	49.90	52.15	50.56	51.73	—

s.e. of difference between any pair of interaction means ± 1.518 degrees
s.e. of difference between means for cut and uncut seeds ± 0.762 ,,
s.e. of difference between any pair of means for Gibrel levels ± 1.074 ,,
min. 5% sig. diff. between means for cut and uncut seeds 1.55 ,,
min. 5% sig. diff. between means for Gibrel levels 2.19 ,,

± 2.387 degrees
± 1.193 ,,
± 1.688 ,,
2.43 ,,
3.44 ,,

10 p.p.m. probably depresses cut seeds significantly, cf. 0 p.p.m. (but highest value on uncut not significant)

10 p.p.m. probably depresses uncut seeds significantly, cf. 1 and 100 p.p.m.
10 p.p.m. probably enhances cut seeds significantly, cf. 0 p.p.m.

In all cases the effect of cutting is significant

TABLE 3 *(Continued)*. EFFECT OF GIBREL SOLUTIONS AND CUTTINGS ON GERMINATION. 5 × 100 SEEDS PER TREATMENT

Cutting treatment	(c) *Flemingia congesta*									
	Percentage germination					Transformed to degrees				
	Gibrel p.p.m.					Gibrel p.p.m.				
	0	1	10	100	Means	0	1	10	100	Means
Uncut	66.8	71.8	68.8	66.8	68.6	54.85	57.97	56.12	54.91	55.96
Cut	86.2	94.4	93.0	92.8	91.6	68.83	76.34	74.76	74.61	73.63
Means	76.5	83.1	80.9	79.8	—	61.84	67.16	65.44	64.76	—

Cutting treatment	(d) *Pueraria phaseoloides*									
	Percentage germination					Transformed to degrees				
	Gibrel p.p.m.					Gibrel p.p.m.				
	0	1	10	100	Means	0	1	10	100	Means
Uncut	34.2	28.8	25.2	29.2	29.4	35.73	32.34	30.06	32.70	32.71
Cut	97.2	97.0	98.2	97.4	97.5	80.71	80.46	83.49	80.87	81.38
Means	65.7	62.9	61.7	63.3	—	58.22	56.40	56.78	56.78	—

s.e. of difference between any pair of interaction means ± 2.253 degrees
s.e. of difference between means for cut and uncut seeds ± 1.126 ,,
s.e. of difference between any pair of means for Gibrel levels ± 1.593 ,,
min. 5% sig. diff. between means for cut and uncut seeds 2.30 ,,
min. 5% sig. diff. between any pair means for Gibrel levels 3.25 ,,

± 2.061 degrees
± 1.031 ,,
± 1.457 ,,
2.10 ,,
2.97 ,,

Gibrel enhances germination, particularly on cut seeds, for which 1 p.p.m. is significant and 10 and 100 p.p.m. are probably significant, cf. 0 p.p.m. Note declining trend with increasing Gibrel concentration.

10 p.p.m. depresses uncut seeds, significantly, cf. 0 p.p.m. (but highest value on cut not significant)

In all cases the effect of cutting is highly significant

TABLE 4. EFFECT OF GIBREL AND KINETIN SOLUTIONS ON GERMINATION OF OTHERWISE UNTREATED SEED, 3 × 100 SEEDS PER TREATMENT

Kinetin p.p.m.	(a) *Calopogonium mucunoides*								(b) *Centrosema pubescens*							
	Percentage germination				Transformed to degrees				Percentage germination				Transformed to degrees			
	Gibrel p.p.m.				Gibrel p.p.m.				Gibrel p.p.m.				Gibrel p.p.m.			
	0	10	100	Means	0	10	100	Means	0	10	100	Means	0	10	100	Means
0	32.8	32.6	33.3	32.9	34.9	34.8	35.3	35.0	33.6	36.3	31.0	33.6	35.4	37.1	33.8	35.4
1	37 3	33.9	32.0	34.4	37.7	35.6	34.5	35.9	31.6	40.3	37.9	36.6	34.2	39.4	38.0	37.2
10	32.6	32.8	37.6	34.3	34.8	34.9	37.8	35.9	34.3	38.7	41.0	37.9	35.9	38.5	39.8	38.0
Means	34.2	33.1	34.3		35.8	35.1	35.9		33.2	38.4	36.6		35.2	38.3	37.2	

s.e. any pair of interaction means ± 1.92 degrees
s.e. any pair of main effect means ± 1.11 ,,
min. 5% sig. diff. of main effect means 3.3 ,,

Germination of cut seed = 84.7% or 66.9 degrees

± 1.47 degrees
± 0.85 ,,
2.5 ,,

Germination of cut seed = 79.0% or 62.8 degrees

TABLE 4 *(Continued).* EFFECT OF GIBREL AND KINETIN SOLUTIONS ON GERMINATION OF OTHERWISE UNTREATED SEED, 3 × 100 SEEDS PER TREATMENT

Kinetin p.p.m.	(c) *Flemingia congesta*								(d) *Pueraria phaseoloides*							
	Percentage germination				Transformed to degrees				Percentage germination				Transformed to degrees			
	Gibrel p.p.m.				Gibrel p.p.m.				Gibrel p.p.m.				Gibrel p.p.m.			
	0	10	100	Means	0	10	100	Means	0	10	100	Means	0	10	100	Means
0	31.0	31.6	33.3	32.0	33.8	34.2	35.3	34.4	67.3	69.1	73.7	70.1	55.1	56.2	59.2	56.8
1	29.0	32.3	29.3	30.2	32.6	34.6	32.8	33.3	75.0	74.5	64.4	71.4	60.0	59.7	53.4	57.7
10	31.6	27.0	30.2	29.6	34.2	31.3	33.4	33.0	66.5	72.4	64.5	67.8	54.6	58.3	53.4	55.4
Means	30.5	30.3	30.9		33.5	33.4	33.8		69.7	72.0	67.6		56.6	58.1	55.3	

s.e. any pair of interaction means ± 1.50 degrees
s.e. any pair of main effect means ± 0.86 ,,
min. 5% sig. diff. of main effect means 2.6 ,,

Germination of cut seed = 91.0% or 72.7 degrees

± 1.81 degrees
± 1.05 ,,
3.1 ,,

Germination of cut seed = 92.3% or 73.9 degrees

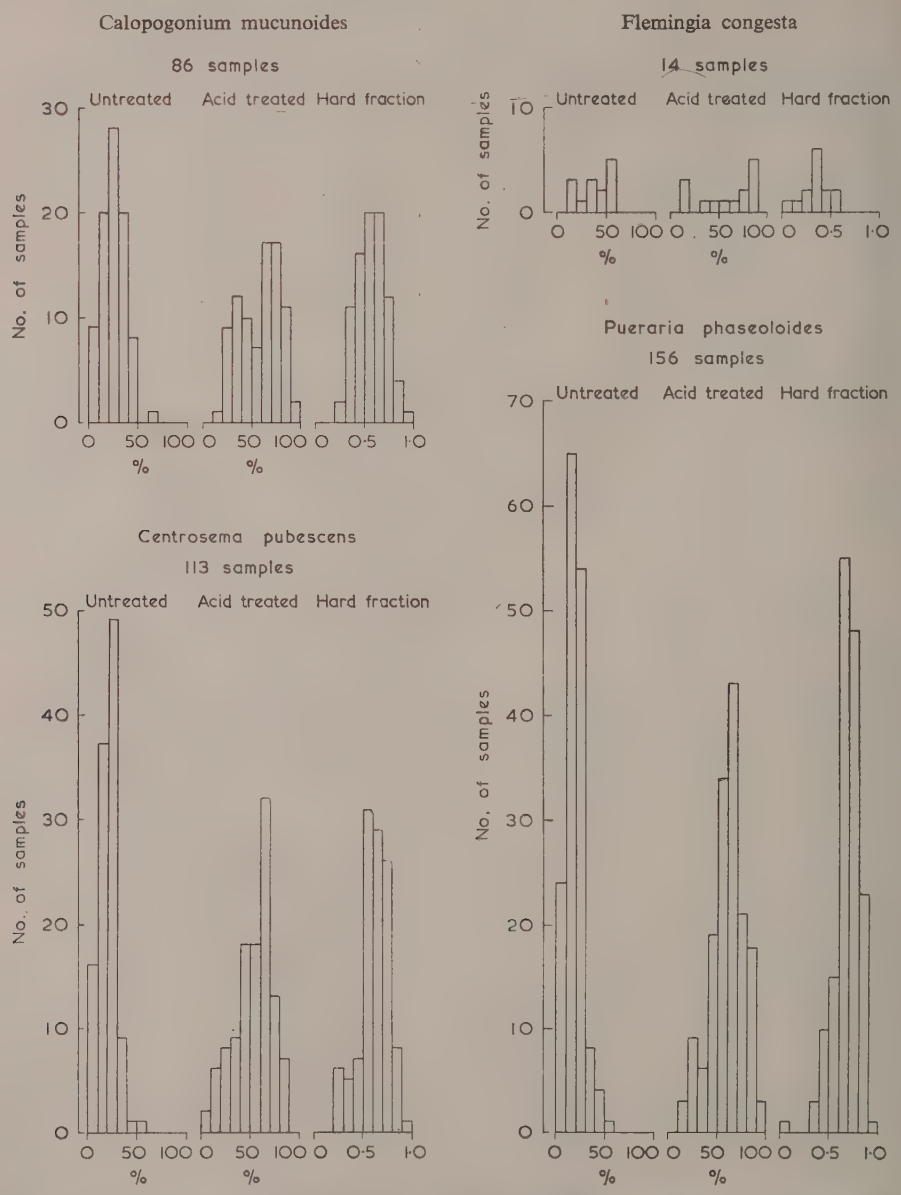


Figure 1. Numbers of commercial samples grouped according to percentage germination with and without acid pretreatment. Also the 'hard seed fraction'. 500 seeds per sample subjected to each test.

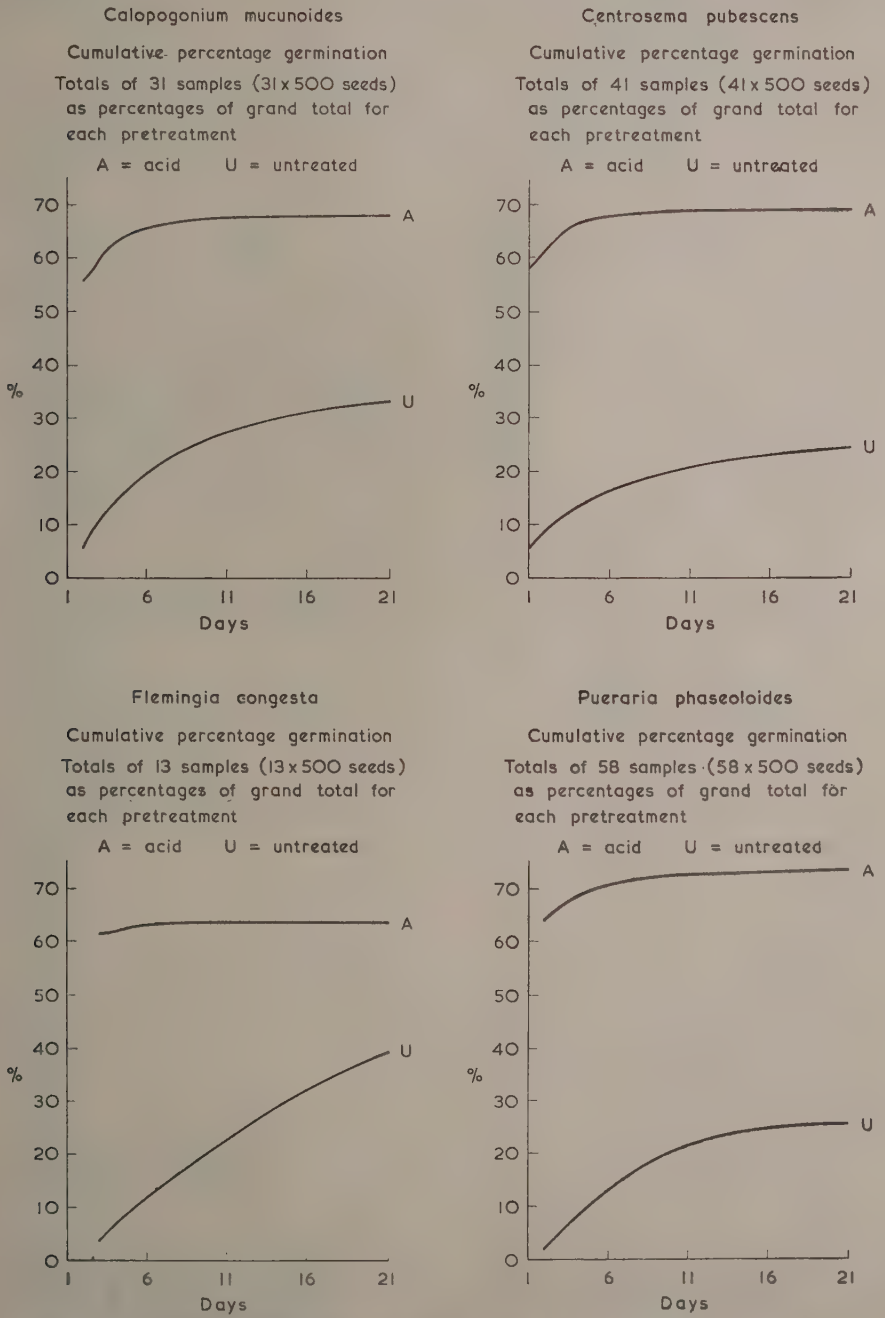

Calopogonium mucunoides
Cumulative percentage germination
Totals of 31 samples (31 x 500 seeds) as percentages of grand total for each pretreatment
A = acid U = untreated
Centrosema pubescens
Cumulative percentage germination
Totals of 41 samples (41 x 500 seeds) as percentages of grand total for each pretreatment
A = acid U = untreated
Flemingia congesta
Cumulative percentage germination
Totals of 13 samples (13 x 500 seeds) as percentages of grand total for each pretreatment
A = acid U = untreated
Pueraria phaseoloides
Cumulative percentage germination
Totals of 58 samples (58 x 500 seeds) as percentages of grand total for each pretreatment
A = acid U = untreated
%
Days
A
U

Figure 2. Cumulative germination over 21 days of untreated and acid treated seed samples.

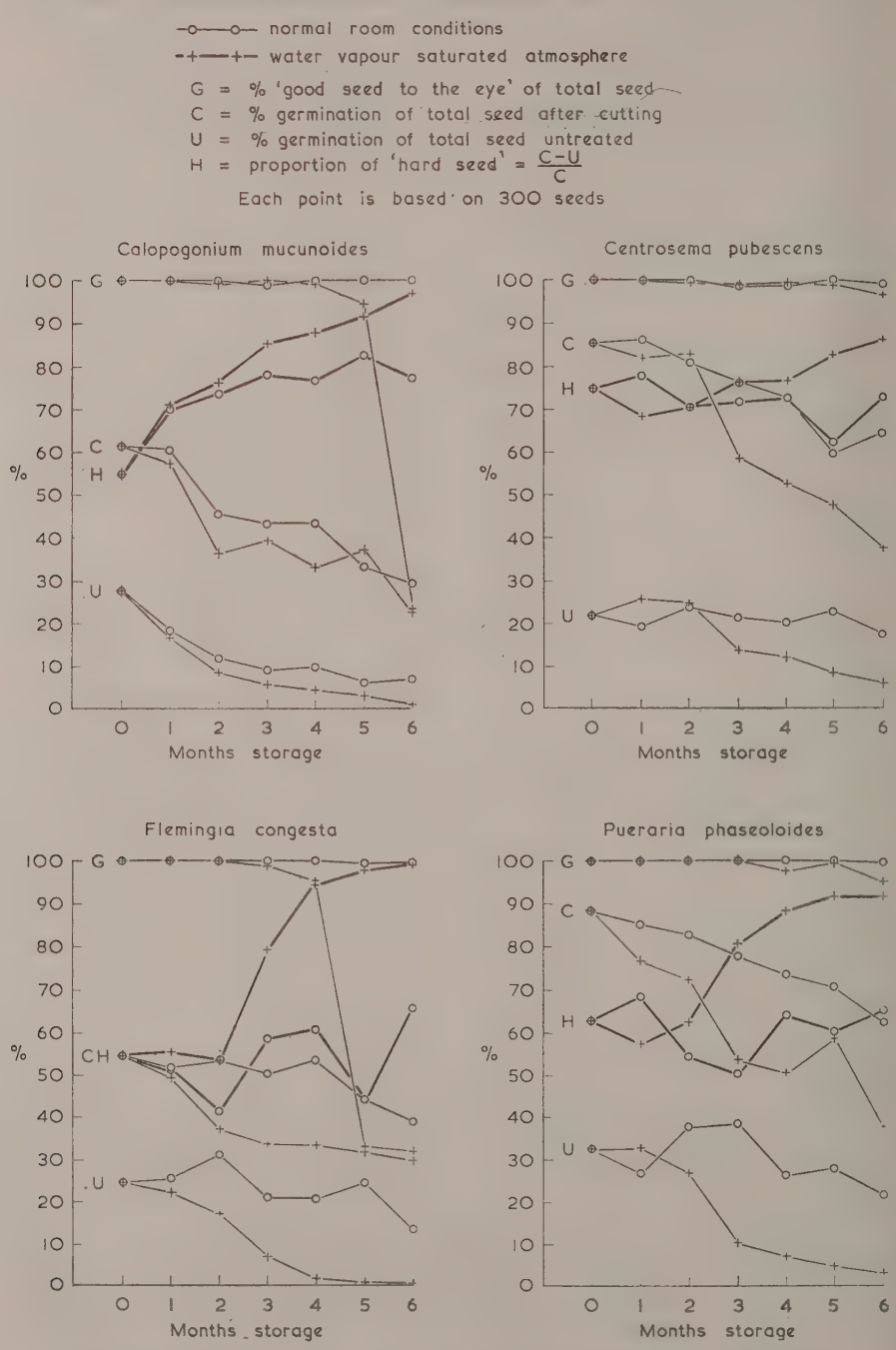


Figure 3. Storage of seed under normal room conditions and in a humid atmosphere.

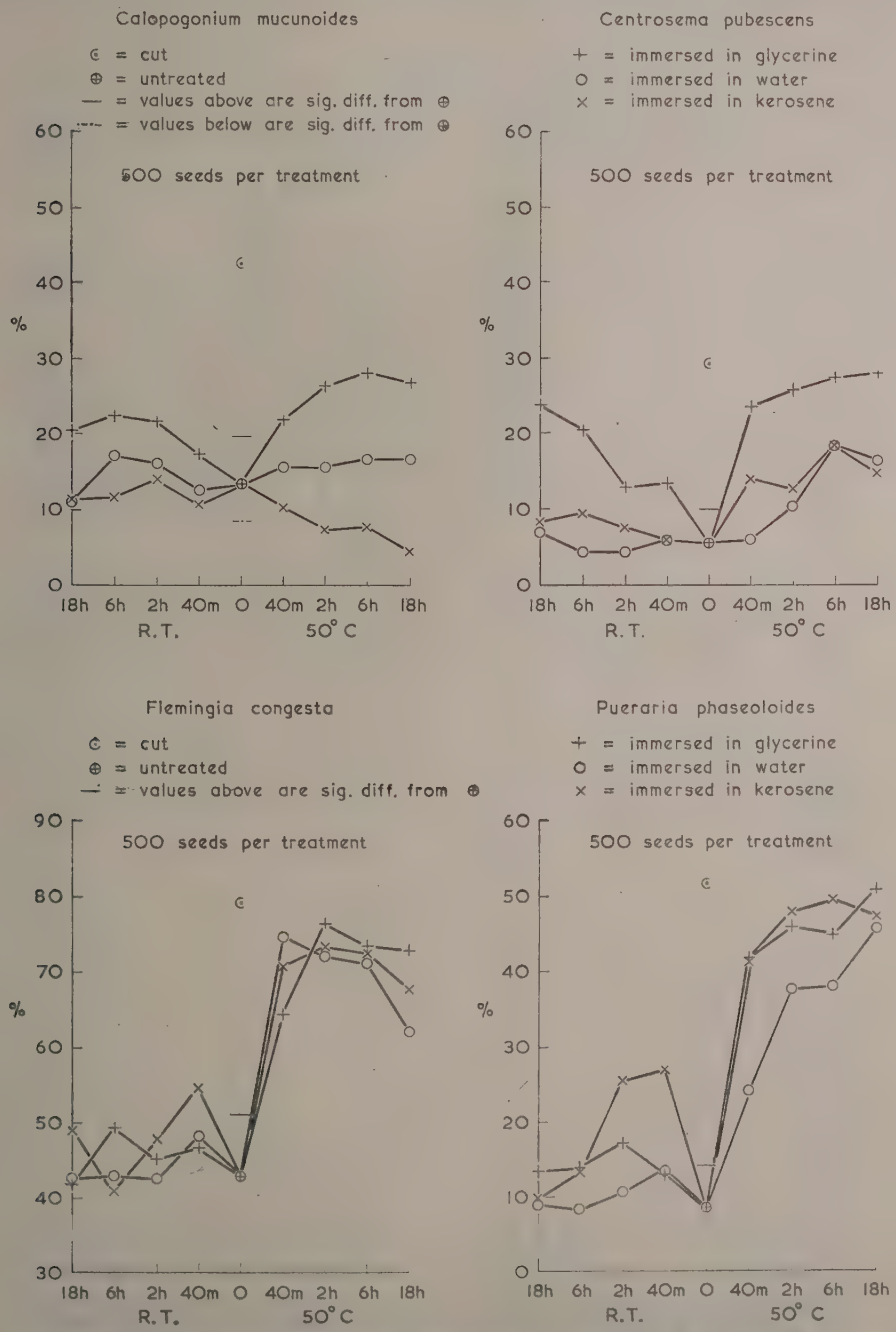

Calopogonium mucunoides
c = cut
⊕ = untreated
— = values above are sig. diff. from ⊕
--- = values below are sig. diff. from ⊕
500 seeds per treatment
%
18h 6h 2h 40m O 40m 2h 6h 18h
R.T. 50° C
Centrosema pubescens
+ = immersed in glycerine
O = immersed in water
× = immersed in kerosene
500 seeds per treatment
%
18h 6h 2h 40m O 40m 2h 6h 18h
R.T. 50° C
Flemingia congesta
c = cut
⊕ = untreated
— = values above are sig. diff. from ⊕
500 seeds per treatment
%
18h 6h 2h 40m O 40m 2h 6h 18h
R.T. 50° C
Pueraria phaseoloides
+ = immersed in glycerine
O = immersed in water
× = immersed in kerosene
500 seeds per treatment
%
18h 6h 2h 40m O 40m 2h 6h 18h
R.T. 50° C

Figure 4. Effect on germination of immersion of seeds in three liquids at two different temperatures.

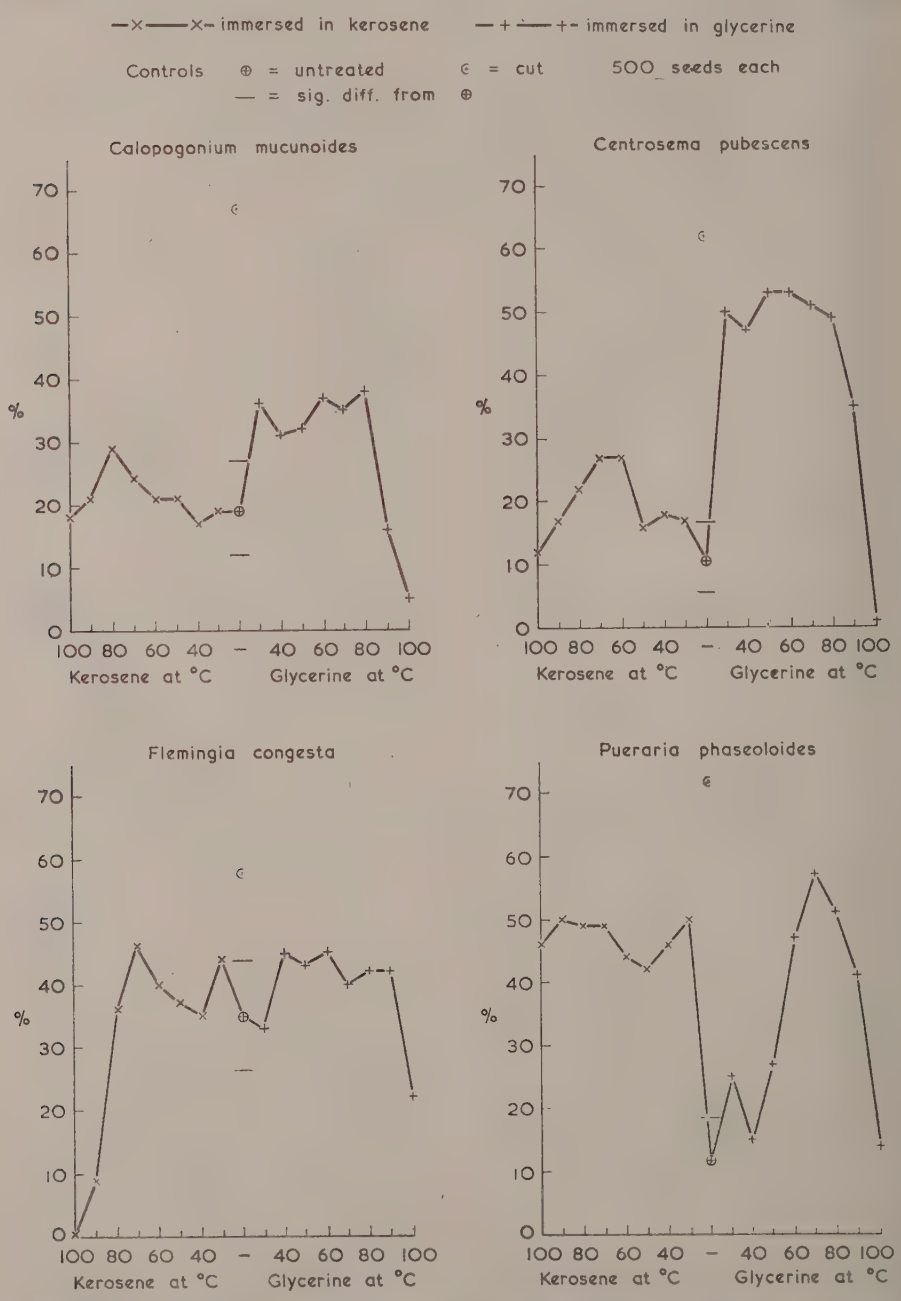

—×——×— immersed in kerosene
—+——+— immersed in glycerine
Controls ⊕ = untreated ⊖ = cut 500 seeds each
— = sig. diff. from ⊕
Calopogonium mucunoides
Centrosema pubescens
Flemingia congesta
Pueraria phaseoloides
%
0 10 20 30 40 50 60 70
100 80 60 40 — 40 60 80 100
Kerosene at °C
Glycerine at °C

Figure 5. Effect on germination of immersion of seeds in liquids at various temperatures followed by cooling in the liquid for 2 hours. 300 seeds per treatment.

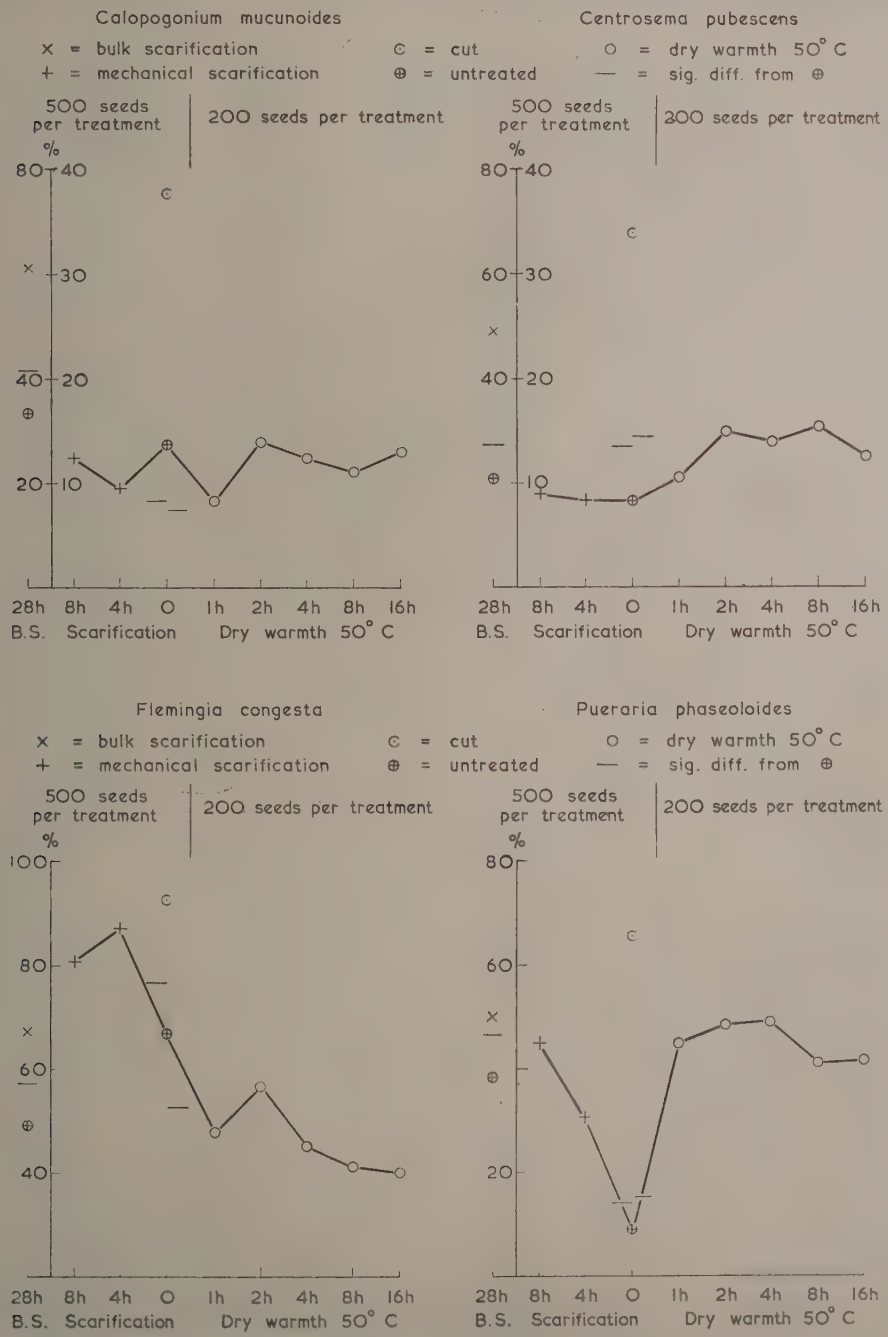


Figure 6. Effects on germination of mechanical scarification and dry warmth. The effects of 28 hours scarification of bulked seed (B.S.) is shown on the left of each graph.

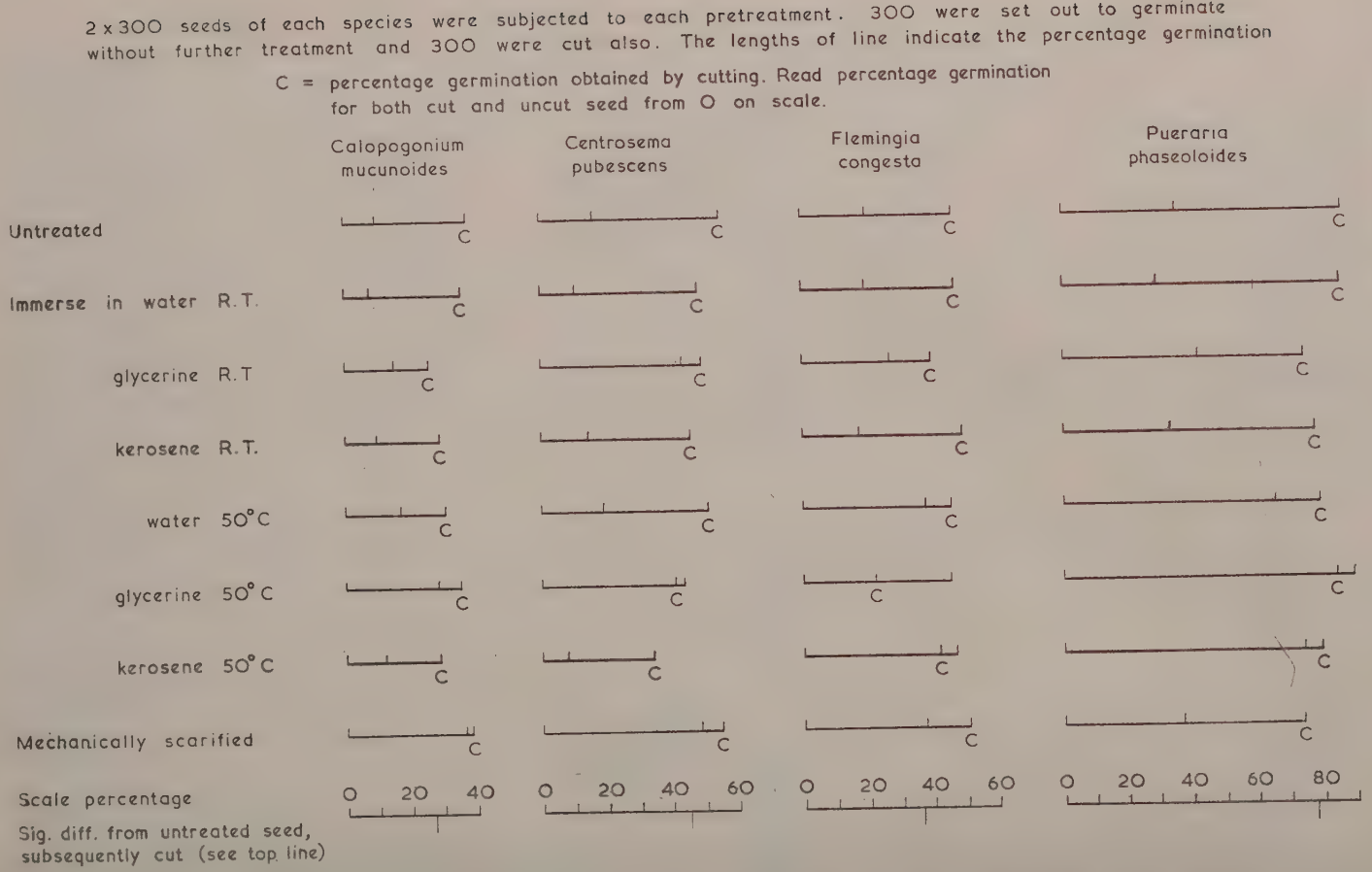

2 x 300 seeds of each species were subjected to each pretreatment. 300 were set out to germinate without further treatment and 300 were cut also. The lengths of line indicate the percentage germination
C = percentage germination obtained by cutting. Read percentage germination for both cut and uncut seed from O on scale.
Calopogonium mucunoides
Centrosema pubescens
Flemingia congesta
Pueraria phaseoloides
Untreated
Immerse in water R.T.
glycerine R.T
kerosene R.T.
water 50°C
glycerine 50°C
kerosene 50°C
Mechanically scarified
Scale percentage
0 20 40
0 20 40 60
0 20 40 60
0 20 40 60 80
Sig. diff. from untreated seed, subsequently cut (see top line)

Figure 7. Percentage germination obtained by various treatments with or without subsequent cutting treatment.

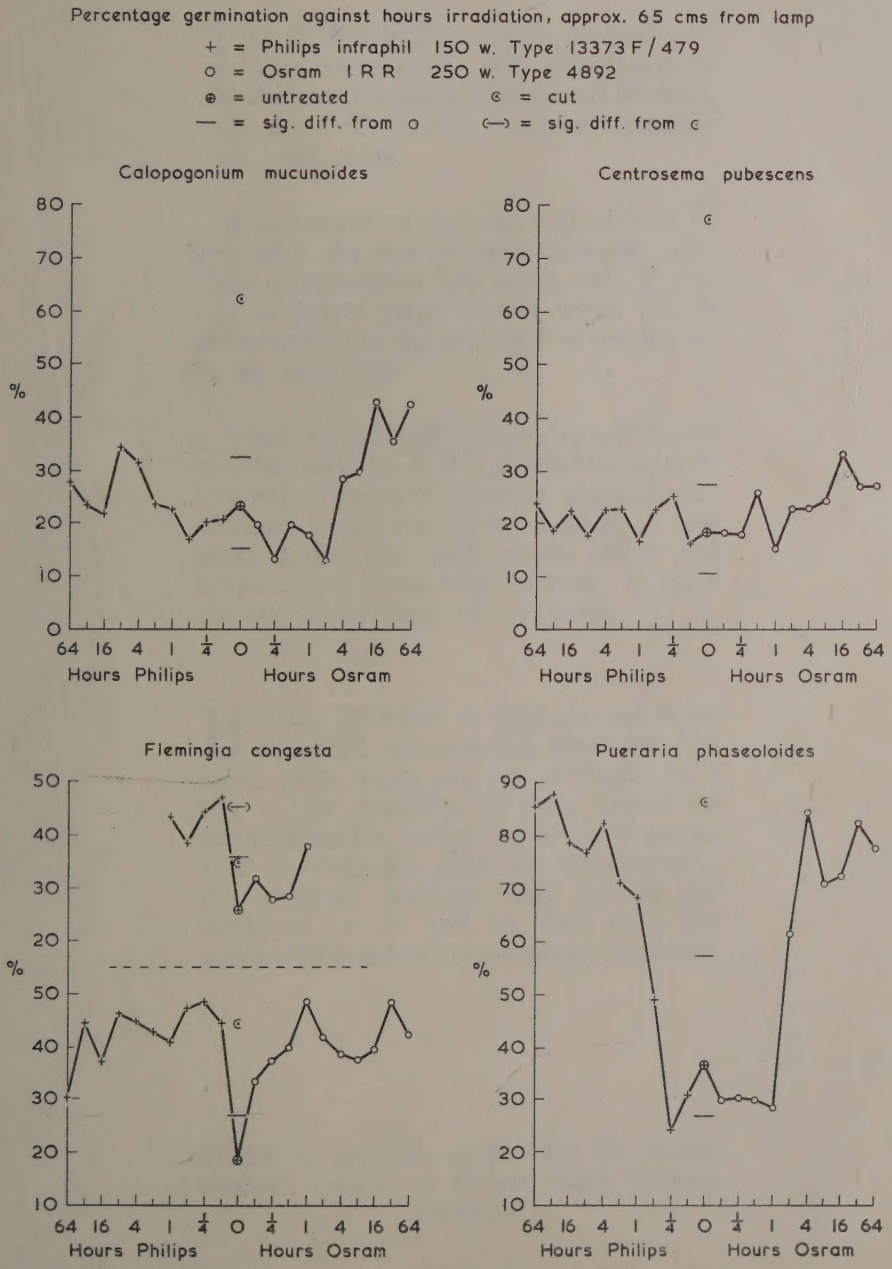
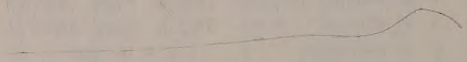

Percentage germination against hours irradiation, approx. 65 cms from lamp
+ = Philips infraphil 150 w. Type 13373 F / 479
o = Osram I R R 250 w. Type 4892
⊕ = untreated
c = cut
— = sig. diff. from o
(—) = sig. diff. from c
Calopogonium mucunoides
Centrosema pubescens
Flemingia congesta
Pueraria phaseoloides
%
64 16 4 1 ¼ 0 ¼ 1 4 16 64
Hours Philips
Hours Osram

Figure 8. Effect of irradiation by infra-red lamps on germination of seeds. 300 seeds per sample.



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